

The Journal of Comparative Pathology and Therapeutics

Vol. 63. No. 2.

Price Seventeen Shillings and Sixpence

1. The columns of the Journal are open for contributions recording original research in veterinary science.

2. The Editorial Board accepts papers bearing on the above subject on the understanding that they are not being offered elsewhere for publication. It reserves the right to reject any papers submitted to it for publication.

3. It is essential that all articles should be concise. Any necessary allusions to matter already published should be brief and limited strictly to those relevant to the satisfactory exposition of the author's own work.

4. Citation of literature in the text should be accompanied by the year of publication. Authors' names (followed by year, publication, etc.) should

be arranged alphabetically in a list at the end of the paper.

5. Papers for publication, exchange journals, etc., should be sent to Prof. T. J. Bosworth, Royal Veterinary College, London, N.W.1, or to any member of the Editorial Board.

6. Correspondence concerning business matters should be addressed to the Publishers, The University Press of Liverpool, Liverpool, 3.

7. The net price of each volume of four numbers is £3 paid in advance to the publishers. Single numbers may be obtained at a price of 17s. 6d. each.

Additional copies and reprints ordered before the issue is printed may be obtained from the publishers.

CONTENTS

	PAGE
Migration Route of <i>Dictyocaulus viviparus</i> and <i>D. filaria</i> Infective Larvae to the Lungs. By K. N. Soliman ..	75
Blood and Visceral Changes in Canine Leukaemia (<i>Lymphadenosis</i>). By A. R. Jennings	85
Leptospirosis in Cattle in Israel. By J. van der Hoeden, Chedva Halevy and I. Dafni	93
Leptospirosis among Goats in Israel. By J. van der Hoeden	101
Studies on the Avian Leukosis Complex. 2. Some Observa- tions on the Behaviour of Three Transplantable Avian Lymphoid Tumours. By C. le Q. Darcel	112
Studies on the Avian Leukosis Complex. 3. A Note on the Maintenance of Avian Lymphoid Tumour Strains. By C. le Q. Darcel	131
An Enrichment Broth and Differential Medium for the Isolation of Mastitis Organisms from the Milk of Distant Herds. By A. G. Innes	136
Outbreak of Nocardiosis in Marsupials in the Brisbane Botanical Gardens. By R. Tucker and R. Miller	143
An Anti-tuberculous Substance in Tuberculous Organs. By M. A. Soltys	147
Terminal or Regional Ileitis in Pigs. By H. I. Field, D. Buntain and A. R. Jennings	153

MIGRATION ROUTE OF *Dictyocaulus viviparus* AND *D. FILARIA* INFECTIVE LARVAE TO THE LUNGS*

By

K. N. SOLIMAN

Veterinary Investigation Department, Reading

INTRODUCTION

CONTRADICTIONARY observations on the migration route taken by the infected larvae of *Dictyocaulus viviparus* and *Dictyocaulus filaria* in reaching the lungs of their hosts have been recorded. During the first thirty years of this century it was commonly accepted that the infective larvae of both species passed *via* the blood stream. Subsequently the view gained ground that the larvae reached the lungs *via* the lymph stream. A review of the relevant literature shows that the migration route of the larvae of both species is still not exactly determined and therefore debatable. Daubney (1920) and Kauzal (1933), who both used guinea-pigs to establish infection in laboratory animals, confined their experiments solely to *D. filaria*. Information on the development of the parasitic phase of the life-cycle of both species is incomplete and more observations are desirable, especially on *D. viviparus*.

The present studies were accordingly made in an attempt to increase the information on these points. They deal with observations on cases occurring in the field and those made on experimental laboratory animals under controlled conditions. In the first part of this investigation, which concerns *D. viviparus* only, material was collected from (a) foetuses, from cows affected with parasitic bronchitis, whether aborted, dead, or killed moribund, to check on pre-natal infection ; (b) bull calves from cows affected in the later stages of gestation with parasitic bronchitis ; (c) faeces from female calves from cows affected with parasitic bronchitis ; (d) livers and mesenteric lymphatic glands of cattle that died or were killed as a result of parasitic lung infestation, especially at the beginning of outbreaks.

METHODS

Only foetuses newly aborted or collected from cows which had just died or recently been killed were examined. The lungs, with the trachea and pharynx intact, were removed ; the pharynx was then severed and using a 50 cc. glass syringe to which was attached a 12 inch length of rubber tubing the bronchial tree was lavaged with physiological saline solution. The washings were then sedimented and the residue examined. The tracheal branches were then opened and examined. Finally the whole lung was minced and extracted with physiological saline solution in a Baermann apparatus at 37°C, for 8 hours.

* Part of a thesis for the degree of Ph.D., University of Reading.

Samples of approximately 200 g. of faeces were collected from the rectum of calves at ten day intervals for over two months and examined by the Baermann technique. Samples of about 25 g. from selected parts of the liver, up to a total of approximately 250 g., were then minced, extracted in the Baermann apparatus and examined. The mesenteric glands were similarly treated.

RESULTS

NATURAL CASES

Observations on Intra-uterine Infection

In view of the contention of certain workers that intra-uterine infection may occur, material was examined as under with the following results :

Twenty-six foetuses, collected from cows suffering from parasitic bronchitis, yielded no *D. viviparus* in any of its stages. Two bull calves newly born to cows suffering from parasitic bronchitis had no lungworm infestation. Sixty faeces samples collected from heifers, born while their dams were suffering from parasitic bronchitis, were all negative for larvae of *D. viviparus*.

Although the number of foetuses and bull calves examined was relatively small, the negative results obtained show that, if intra-uterine infection does occur, it cannot be considered as a common occurrence.

The life-cycle in cases occurring under natural conditions in the field

To try to follow the route the infective stage of *D. viviparus* take in reaching the lungs of their hosts, the cases chosen were animals which were the first to die in outbreaks or were still grazing an infected field and thus still ingesting infective material. The mesenteric lymphatic glands, especially those showing oedema or congestion, were collected.

Table I shows the results from the seventeen cases examined. No larvae were found in the liver either in press preparations or in material from Baermann extractions, but they were present in very variable numbers in the mesenteric glands and when found alive, were in the third, unsheathed active stage. Ensheathed larvae were, however, observed in press preparations on three occasions in cases No. 2, 6 and 13 although examination of the same material by the Baermann technique was negative. These findings suggest that the active larvae had passed on to the lungs leaving behind a few dead ensheathed ones. In none of the cases were ensheathed live larvae observed in the mesenteric glands.

These observations, coupled with the negative findings regarding pre-natal infection, strongly indicate the mesenteric lymphatic channels as being the route that *D. viviparus* larvae follow to reach the lungs of their hosts.

TABLE I

OCCURENCE OF *D. viviparus* LARVAE IN LYMPHATIC GLANDS FROM
CASES OCCURRING UNDER NATURAL CONDITIONS IN THE FIELD

Case No.	Date	Breed	Age		Result of Examination	
			yrs.	months	Mesenteric lymph glands	
					Press preparations	Baermann extraction
1	20. 7.48	Shorthorn	—	8	Exsheathed active third stage	Exsheathed active third stage
2	22. 7.48	Guernsey	—	8	Few ensheathed—seemingly dead	Negative
3	25. 8.48	Jersey	—	1	Negative	Few exsheathed third active stage
4	13. 7.49	Jersey	—	6	Few exsheathed active third stage	Few exsheathed active third stage
5	1. 9.49	Shorthorn	—	8	Negative	Negative
6	3.11.49	Guernsey	—	8	Few ensheathed dead larvae	Negative
7	9. 8.50	Ayrshire	3	—	Few ensheathed third stage active	Few exsheathed third active stage
8	9. 8.50	Ayrshire	3	—	Negative	Few exsheathed third active stage
9	10. 8.50	Ayrshire	3	—	Negative	Negative
10	12. 8.50	Guernsey	—	8	Negative	Negative
11	24. 9.50	Guernsey	—	8	Negative	Negative
12	16. 7.51	Guernsey cross	—	6	Few exsheathed third active stage	Few exsheathed third active stage
13	4. 8.51	Guernsey cross	—	6	Few ensheathed dead larvae	Negative
14	21.10.50	Shorthorn	2	—	Few exsheathed third active stage	Few exsheathed third active stage
15	24.10.51	Shorthorn	2	—	Negative	Negative
16	29.10.51	Shorthorn	3	—	Negative	Negative
17	29.10.51	Shorthorn	3	6	Negative	Negative

EXPERIMENTAL INFECTIONS IN SMALL LABORATORY ANIMALS.

This part of the study is complementary to the field observations previously mentioned. The main object was to investigate, under controlled laboratory conditions, the route travelled by the infective larvae in reaching the lungs of their hosts and thus to follow the

parasitic phase of the life-cycle. Rabbits, guinea-pigs and white mice were experimentally infected with both *D. filaria* and *D. viviparus* larvae. The material was collected, as first stage larvae, from the faeces of heavily infected sheep and cattle by the Baermann extraction technique. The larvae, after being washed several times, were kept at room temperature (approximately 20°C.) in ordinary tap water through which air was bubbled continuously for seven days to ensure a high survival rate and development to the third infective stage.

Life-cycle of D. filaria

Rabbits. Two rabbits were each dosed with 10,000 third stage larvae and autopsied two and five days respectively after administration. In the first rabbit, active third stage larvae, measuring about 555 μ in length by 19 μ in breadth, were observed in the mesenteric glands. The liver and lungs were normal and no larvae were detected in them. Similar results were obtained in the mesenteric glands of the second rabbit, the liver of which was normal and yielded no larvae: the lungs, however, showed separate areas of congestion and larvae in the active third stage were observed.

From this experiment, it would appear that between the time of infection and the 48th hour the larvae pass through the lymphatic glands and arrive in the lungs between the second and the fifth days after infection.

Guinea-pigs. Fourteen guinea-pigs, 6 to 7 weeks old, were used. Seven were each dosed with 7,000 third stage *D. filaria* larvae and were autopsied, along with a control animal, at 24 hours, 3, 7, 15, 20, 22 and 24 days respectively after administration. No larvae were found in the livers of six of the guinea-pigs, but press preparations of the liver of the seventh animal, autopsied 20 days after infection, revealed two dead female worms in the fourth stage coiled round each other. The mesenteric lymph nodes of the guinea-pigs examined after 24 hours, 3 and 7 days were slightly congested and yielded larvae in the active third stage measuring about 560 μ in length by 19 μ in breadth. The mesenteric lymph glands of the four guinea-pigs were free from larvae.

The lungs of the guinea-pig autopsied 24 hours after infection were normal and no larvae were detected in them, but those of the second animal killed 3 days after infection contained active third stage larvae. The lungs of the guinea-pig killed at 7 days exhibited discrete areas of congestion and had fourth stage sexually differentiated larvae in the bronchi. The males were 580 μ in length by 21 μ in breadth, each having a well-developed bursa that showed rays, with the exception of the ventrals, and no spicules. Females were 600 μ in length by 26 μ in breadth. The lungs of the guinea-pig autopsied 15 days after infection showed pneumonic patches at the fringe of the lobules at the point of bifurcation of the main

branches, and contained fifth stage larvae in the bronchi. Males measured 3 to 5 mm. long by 100μ in breadth, each with a well-developed bursa, showing all rays, and developed spicules. Females measured 4 to 6 mm. in length by $100-120\mu$ in breadth and the openings of the vulvae were patent. The lungs of the animal autopsied at 20 days exhibited pneumonia at the apical, cardiac, intermediate and base of the diaphragmatic lobes. A number of young adult worms were found in the bronchi, the males ranging from 8 to 10 mm. long each with a well-developed bursa and prominent spicules, and the females ranging from 8 to 12 mm. long. The lungs of the guinea-pig autopsied at 22 days were pneumonic, showing lesions similar to those met with in cattle, and in which the apical, cardiac and intermediate lobes were almost completely involved on both sides as well as the base of the diaphragmatic lobes. From the bronchi eighteen specimens were collected. The 7 males varied between 8 and 17 mm. long by 136 and 195μ wide; their spicules were between 130μ and 260μ long. The bursal rays were all showing normally except in one specimen in which the medio- and externo- laterals were missing possibly because the parasite was in an abnormal host. The 11 female worms varied between 7 and 15 mm. long by 130 and 200μ broad, the vulva being about 8 mm. from the head end.

The lungs of the guinea-pig killed at 24 days showed the same features as those observed in the previous animal and 4 male and 9 female worms were collected from the bronchial tubes.

TABLE II

SUMMARY OF THE RESULTS OF EXPERIMENTAL INFECTIONS WITH
Dictyocaulus filaria IN GUINEA-PIGS

Guinea-pig No.	Interval between inoculation & autopsy	Results of Examination				
		Liver		Lymphatic Glands		Lungs
		Press Prep.	Baermann	Press Prep.	Baermann	
1	24 hours	—	—	+	+	—
2	3 days	—	—	+	+	+ in Baermann
3	7 days	—	—	+	+	+ 4th stage
4	15 days	—	—	—	—	+ 5th stage
5	20 days	2 females coiled in 4th stage	—	—	—	+ young adults
6	22 days	—	—	—	—	+ 19 young adults
7	24 days	—	—	—	—	+ 15 young adults

Post-mortem examination of the seven control guinea-pigs revealed no abnormalities and no parasites were encountered. Table II summarizes the results of this experiment and provides evidence of the route that is taken to reach the lungs.

The experimental guinea-pigs were observed daily. An interesting feature of this experiment was that, on the 14th day after infection, they started to sneeze and Nos. 5, 6 and 7 exhibited a wheezing cough similar to that observed in cattle with parasitic pneumonia. The respiration was laboured. These symptoms most probably were due to the pneumonia, first noticed in guinea-pig No. 4 killed 15 days after infection, which was beginning to involve the fringe of the different lobes at the bifurcation of the main bronchii. It was intended to leave guinea-pig No. 7, in the hope of finding if the parasites would develop to full maturity and start reproducing but, unfortunately, the animal died as a result of pneumonia.

Mice. Twenty mice, 6 to 7 weeks old, were each fed 3,000 infective larvae of *Dictyocaulus filaria* and another batch of twenty animals were kept as controls. They were autopsied at regular intervals after infection, one mouse being killed every 24 hours up to 20 days. The main objects were again to explore the route of larval migration from the digestive tract to the lungs, the time this migration took and, further, to follow the development of the fourth and fifth stages of the parasite.

No larvae were found in the livers of the 20 mice. Third active stage larvae were seen in the mesenteric glands in both press preparations and Baermann extraction samples 24 hours after the feeding the infective larvae, but on the sixth day there were only a few found. After the seventh day no larvae were detected in the mesenteric glands, which were normal in appearance, with the exception of a few ensheathed infective larvae in press preparations from mice Nos. 9 and 10.

The lungs of mice Nos. 1 and 2 were normal and contained no parasites. The lungs of the remaining mice exhibited a slight, discrete congested area and in animals killed on and after the 15th day there was definite pneumonia in the apical, cardiac, intermediate and anterior part of the base of the diaphragmatic lobes on both sides. Larvae first appeared in the lungs on the third day in small numbers. Fourth stage larvae, which could be sexually differentiated, were observed on the eighth day and fifth stage larvae on the fourteenth day.

Post-mortem examination of each of the respective control mice showed no abnormalities and no parasites were encountered.

Life-cycle of D. viviparus

Rabbits. Two rabbits were each dosed with 10,000 third stage larvae and autopsied two and five days respectively after infection. The results were similar to those recorded in *D. filaria* infection.

Larvae, measuring 340μ in length by 18μ in breadth, were found in the mesenteric lymph nodes but none in the livers. They were also observed in the lungs of the rabbit killed five days after infection.

Guinea-pigs. Twenty guinea-pigs, 6 to 7 weeks old, were used. Ten were each dosed with 7,000 third stage larvae of *D. viviparus*, and autopsied along with their respective control animals at 24 hours, 2, 3, 5, 8, 10, 14, 15, 20 and 35 days after infection. The livers of all the animals were normal and no larvae were detected in them. The mesenteric lymph glands and lungs of the first five experimental guinea-pigs exhibited features similar to those observed in the *D. filaria* infection, excepting differences of measurements related to the species. In the sixth guinea-pig, killed 10 days after infection, only one larva was detected in the mesenteric gland, but the lungs showed slight congestion and 32 worms were collected from the bronchial branches. In the males, the bursa with all its rays could be seen as well as the spicules, and in the females the opening of the vulva was showing but could not be described as patent. Of the remaining four guinea-pigs the mesenteric glands were free from larvae and the lungs gave findings similar to those observed in *D. filaria* infection except that the pneumonia and congestion were of less extent.

Post-mortem examination of the ten control guinea-pigs showed no abnormalities and no parasites were encountered.

TABLE III

SUMMARY OF THE RESULTS OF EXPERIMENTAL INFECTION WITH
Dictyocaulus viviparus IN GUINEA-PIGS

Guinea-pig No.	Interval between inoculation & autopsy	Results of Examination		
		Lymphatic Glands		Lungs
		Press. Prep.	Baermann	
1	24 hours	+	+	—
2	2 days	+	+	—
3	3 days	+	+	+
4	5 days	+	+	+
5	8 days	—	—	4th stage and sexes clear
6	10 days	—	—	Immature adults
7	14 days	—	—	Immature adults
8	15 days	—	—	Immature adults
9	20 days	—	—	Immature adults
10	35 days	—	—	Immature adults

The animals were observed daily and an interesting feature of this experiment was that, in contrast to the animals with *D. filaria* infection, none showed any apparent symptoms. There was no sneezing or laboured respirations. The only difference in the diet was that guinea-pigs with the *D. viviparus* infection were receiving supplementary green food in the form of kale leaves.

Table III summarizes the results of this experiment and provides evidence of the route *D. viviparus* larvae take to reach the lungs of their hosts. The results suggest that either guinea-pigs are more susceptible to *D. filaria* than to *D. viviparus* or that the additional quantity of Vitamin A, supplied *via* the carotene of the kale, prevented a desquamation of the respiratory epithelium and thereby reduced the severity of the attack and of the symptoms as compared with the *D. filaria* group.

Mice. Ten mice, six to seven weeks old, were each fed 3,000 infective larvae of *Dictyocaulus viviparus*, another ten mice being kept as controls. They were autopsied at 24 hours, 2, 3, 4, 5, 8, 10, 15, 20 and 25 days after infection. The purpose of the experiment was again to trace the migration route of the larvae from the digestive tract to the lungs, to record the time this migration took and to determine when the fourth and fifth development stages appeared. No larvae were recovered from the livers of the ten mice. Active third stage larvae were seen in the mesenteric lymph glands 24 hours after feeding the infective material and only a few such larvae were present on the sixth day. From the eighth day onwards the glands were negative for active larvae and normal in appearance and after the tenth day retained no larvae.

Larvae were first seen in the lungs after three days and their numbers later increased. Fourth stage larvae, showing distinct sex differentiation, were observed on the eighth day, and fifth stage larvae, in which the opening of the vulva was patent, were seen on the 15th day after infection.

Post-mortem examinations of the respective control mice showed no abnormalities and no parasites were encountered.

DISCUSSION

The only two records that could be traced in the literature dealing with pre-natal infection were those by Kasperek (1900), reporting calves one and one-half to eight days old infected with "lungworm diseases" and by Neveu-Lemaire (1912) who reported the finding of adult *D. filaria* in the foetus *in utero* and in a four days old lamb. These two records appear to relate to cases of accidental infections and are presumably of rare occurrence. The results of examining 26 foetuses, two calves 10 and 35 days old, and faecal samples from ten female calves all from cows affected with parasitic pneumonia support this view. While the numbers examined were not large the consistently negative results obtained point to the

conclusion that the blood stream cannot be considered as the normal route by which *D. viviparus* larvae travel to the lungs. This is supported by the work of Porter and Cauthen (1942) who failed experimentally to establish prenatal infection. The only feasible explanation of these early records seems to be that suggested by Monnig (1949) who postulated that the larvae, after reaching the lungs, may accidentally gain access to the general systemic circulation from the pulmonary capillaries and thus be carried to the foetus. Kauzal (1933) who studied the life history of *D. filaria* to try and confirm the findings of Hobmaier and Hobmaier (1929) recorded the appearance of whitish-grey spots, 0.5 to 1 mm. in diameter, in the liver of a guinea-pig autopsied 48 hours after infection. In these spots he found *D. filaria* larvae in the active third stage and, thus was unable to determine the route the parasite followed in the guinea-pig. No larvae were found in the livers of lambs; in addition, 17 samples of bovine livers from early cases of parasitic pneumonia were free from *D. viviparus* larvae.

In the experimental infections of rabbits, guinea-pigs and mice by both parasites, on one occasion only, a case of *D. filaria* infection, two dead females as fourth stage larvae were found in the liver of a guinea-pig killed 20 days after infection. This confirms Kauzal's view that sometimes larvae may pass accidentally to the portal circulation. The negative findings in all the livers of the rabbits, guinea-pigs and mice support the view that the larvae follow the lymph channels. Although Kauzal (1933) found no larvae in the lymph glands of four experimentally infected guinea-pigs, in the experiments recorded here third stage larvae were observed in the mesenteric lymph glands of all the animals 24 hours after infection and generally were continuously observed up to 5 or 6 days and, on rare occasions, on the tenth day after infection.

The guinea-pig might be a satisfactory animal for artificial infections with *D. filaria* and *D. viviparus* and might be of use in the experimental treatment of both parasites.

SUMMARY

Twenty-six foetuses, two calves 10 and 35 days old respectively from cows suffering from parasitic bronchitis, and faecal samples from 10 calves born of cows suffering from lungworm diseases were all negative for any stage of *D. viviparus*. An examination of 17 livers from cattle affected with lungworms did not reveal any parasites, but they were detected in some mesenteric lymph glands from the same animals.

In experimental infections of rabbits, guinea-pigs, and mice with infective larvae of *D. filaria* and *D. viviparus* the migratory route of the larvae and parasitic phase of the life-cycle of both species were followed. Infective larvae of both species were found after 24 hours in the mesenteric lymph glands and in the lungs

on the third day. The active third stage larvae passed through the lymphatic glands during the first five to six days. Larvae had developed to the fourth stage on the 6th day, and the sexes were clearly differentiated. Fifth stage parasites were observed at the earliest 14 days after infection. After 22 days the adult female worms showed the vulvar opening clearly and the males showed spicules of normal size.

Pneumonic lesions developed in the same situations as those observed in cattle.

ACKNOWLEDGMENT

Thanks are due to Dr. N. S. Barron for providing facilities to carry out this work and to members of his staff for technical help.

REFERENCES

- Daubney, R. (1920). *J. comp. Path.*, **33**, 225.
Hobmaier, A., and Hobmaier, M. (1929). *Munch. tierarztl. Wschr.*, **80**, 621.
Kasperek, T. (1900). *Arch. wiss. prakt. Tierheilk.*, **26**, 70. (Cited by Daubney and by Porter & Cauthen).
Kauzal, G. P. (1933). *Aust. vet. J.*, **9**, 20.
Monnig, H. O. (1949). *Veterinary Helminthology and Entomology*. London; Bailliére, Tindall & Cox, 3rd Ed.
Neveu-Lemaire, M. (1912). *Compte R. Acad. Sci., Paris*, **154**, 1311.
Porter, D. A., and Cauthen, G. E. (1942). *Amer. J. vet Res.*, **3**, 395.

[Received for publication, 31st July, 1952]

BLOOD AND VISCERAL CHANGES IN CANINE
LEUKAEMIA (LYMPHADENOSIS) *

By

A. R. JENNINGS

Department of Veterinary Pathology, University of Liverpool

INTRODUCTION

ALTHOUGH leukaemia in dogs is not an uncommon disease, there has been a good deal of confusion in the literature with regard to terminology and there are very few records of exact haematological and histological examinations. The purpose of the present investigation is to record the detailed findings in eight cases of the disease.

Siedamgrotzky (1871) was the first to mention leukaemic lymphogenous leukaemia and since that date there have been many records. In 1918, Dahlström and Henschen collected from the literature details of 50 cases; later, a good general review was given by Wirth (1931). Much of the continental literature has been summarised by Hutyra, Marek and Manninger (1938) who subdivided canine lymphatic leukaemia into the following groups; (a) lymphatic leukaemia with a lymphatic leukaemic blood picture, (b) lymphatic leukaemia with no changes in the blood picture (aleukaemic or subleukaemic leukaemia), and (c) lymphatic leukaemia with a leucocytotic blood picture. Engelbreth-Holm (1942) reviewed the leukaemias in animals but dealt with the canine disease in very general terms. He stated that "lymphogenous leukaemias in dogs have somewhat peculiar features and two different types of the disease occur." He also considered that one form corresponds to the disease as seen in man and other animals, while another has the same visceral changes but shows changes in the blood which are non-specific. The typical lymphogenous leukaemia may occur with or without a leukaemic blood picture. With the leukaemic cases there may be up to 600,000 white corpuscles per c.mm. From these descriptions it is clear that Engelbreth-Holm agrees with the classification of Hutyra, Marek and Manninger. Innes, Parry and Berger (1946) considered that the disease in the dog "is complicated by the occurrence of a peculiar form with the same gross visceral pathology but in which the cellular changes are those of a non-specific polymorphonuclear leucocytosis." The previous year Bloom and Meyer (1945) investigated a series of cases and found "it difficult to justify the term leukaemia." They stated that the characteristic blood change, in addition to the anaemia, was a nonspecific leucocytosis. In one case there was a leucopenia. Bloom and Meyer considered that changes in the blood suggestive of acute or chronic lymphatic leukaemia, as seen in man, were absent in the canine cases they investigated, and that the term

* Part of a thesis for the Degree of M.V.Sc. University of Liverpool.

"malignant lymphoma" rather than "leukaemia" should be applied to these cases.

It is doubtful whether the few cases recorded in the literature as myeloid leukaemia were indeed examples of this disease: the data, to the writer, did not justify this term. Engelbreth Holm (1942), Coffin (1945) and Innes, Parry and Berger (1946) are all in agreement on this point.

MATERIALS AND METHODS

The haematological techniques used have been described in an earlier paper (Jennings, 1952). Dogs which died or were killed were examined as soon as possible after death and representative tissues taken and fixed in formol saline and Zenker's fluid. Paraffin sections were stained by haematoxylin eosin, Giemsa and Heidenhain's haematoxylin.

Eight cases of the disease were investigated and a total of 19 blood samples examined. Six of the dogs were Scotch terriers, one a fox terrier and one a collie type dog. All the animals were between three and seven years of age, five were males and three females. Five of the dogs came to post-mortem examination.

RESULTS

The Blood Changes

The changes in the cellular elements of the peripheral blood may be seen in Table I, and the mean values and standard deviations of the series as a whole in Table II.

Analysis of the data indicates that the mean red cell count is at the lower end of the normal range, the haemoglobin figure is below normal as is the volume of packed cells. The mean corpuscular volume (M.C.V.) calculated from these means is 61.87 cubic micra and the mean corpuscular haemoglobin concentration (M.C.H.C.) is 28.87 per cent. These figures indicate a normocytic normochromic anaemia for the series. Individual cases, however, show more severe anaemia, e.g. No. 1, a Scotch terrier, with M.C.V. 84.11 cubic micra and M.C.H.C. 22.22 per cent. In this case, therefore, there is a macrocytic hypochromic anaemia. Clinically this dog which was in the terminal phase of the disease, was emaciated and hidebound. There had been diarrhoea for several days, some loss of appetite and laboured respirations. All the superficial lymph nodes were very prominent and measured up to 3 inches in length. The liver and spleen were easily palpable and extended well beyond the costal arch. The dog died the day after the blood sample was taken.

The mean white cell count for the series, 13.05 ± 4.67 thousands per c.mm., was within the normal range. The highest count of 25,000 cells per c.mm. recorded in the series (Case No. 1) was outside the normal range but indicated leucocytosis and not leukaemia.

TABLE I

No.	T.	RBC.	PCV.	Hb.	WBC.	N.	L.	M.	E.	B.
1	1	3.21	27.0	6.0	25.0	80	16	3	1	0
2	1	5.0	25.0	8.0	12.0	75	13	10	2	0
3	1	6.56	38.0	14.0	10.0	64	29	3	4	0
	14	5.07	33.0	10.0	4.5	60	30	10	0	0
	17	5.0	34.0	11.0	6.0	65	30	5	0	0
	19	5.62	32.5	10.0	6.75	73	21	4	2	0
4	1	4.61	27.0	7.9	15.6	64	30	3	3	
	7	4.46	26.5	7.5	17.2	66	25	3	6	0
	8	4.5	27.0	7.8	15.2	65	30	3	2	0
5	1	3.26	—	6.8	20.0	25	70	2	3	0
6	1	5.6	—	7.91	12.0	35	65	0	0	0
7	1	6.5	30.0	9.5	12.0	64	29	4	3	0
	8	12.0	35.0	11.2	11.5	66	27	4	3	0
8	1	4.5	—	8.8	17.2	50	38	7	5	0
	8	4.39	—	8.6	13.25	44	44	5	7	0
	15	4.35	—	8.8	13.9	49	37	5	9	0
	20	4.6	—	9.1	14.0	47	40	6	7	0
	26	2.9	—	7.3	9.0	45	45	3	4	0
	31	4.1	—	8.0	13.0	45	35	10	5	5

No. = No. of case.

T = Time, in days, when sample taken after dog first seen.

RBC = Red cell count millions per c.mm.

PCV = Packed cell volume (per cent)

Hb = Haemoglobin g. per cent.

WBC = White cell count in thousands per c.mm.

N = Neutrophil (per cent)

L = Lymphocyte (per cent)

M = Monocyte (per cent)

E = Eosinophil (per cent)

B = Basophil (per cent)

TABLE II

	<i>Leukaemic Dogs*</i>	<i>Normal Dogs†</i>
Red Cell Count	5.01 ± 1.91 ⁻⁶ per c.mm.	6.1 ± 1.0 ⁻⁶ per c.mm.
Haemoglobin	8.95 ± 1.92 g. per cent.	13.0 ± 1.8 g. per cent.
Packed Cell Volume ..	31.0 per cent.	39.0 ± 5.8 per cent.
White Cell Count ..	13.05 ± 4.67 ⁻³ per c.mm.	11.3 ± 3.3 ⁻³ per c.mm.
Neutrophils	59.64 ± 13.9 per cent.	74.0 ± 7.0 per cent.
Lymphocytes	34.42 ± 13.9 per cent.	20 ± 5.4 per cent.
Monocytes	4.42 ± 3.04 per cent.	4.0 ± 3.0 per cent.
Eosinophils	3.47 ± 2.47 per cent.	2.0 ± 2.0 per cent.

* Including dogs which had been treated with urethane and nitrogen mustard.

† As given by Holman (1944).

Although most of the dogs had normal white cell counts, they were well-marked clinical cases which had been showing signs of the disease for several weeks. Cases Nos 5 and 6 exhibited a relative lymphocytosis with 70 and 65 per cent. lymphocytes. The remaining six cases showed no marked deviations from normal in regard to cell types. In some of the cases there was a trend towards neutrophilia. In none of the eight cases were primitive white cells seen in the peripheral blood stream.

Pathology

Macroscopic Changes. It is not proposed to deal with the five cases individually since all of them showed a remarkable uniformity. Such deviations as were present were differences in degree only.

The superficial lymph nodes were all enlarged and easily seen and/or palpated. Individual lymph nodes measured up to 3 inches by 2 inches by 1 inch. They were firm and smooth, and usually freely moveable, but in two cases the popliteal lymph nodes were not easily moved and were surrounded by a yellow, gelatinous fluid. On incision the enlarged nodes were greyish-yellow and diffuent. It was not possible to identify cortex and medulla. In the dogs with adherent popliteal lymph nodes section revealed areas of semi-liquefaction and a few scattered haemorrhages. The tonsils in all five dogs were enlarged; in one case (No. 5), the enlargement was of such a nature as to impede swallowing.

There were varying degrees of enlargement of the spleen. In some cases the organ was up to 4 and 5 times the normal weight and extended into the pelvic cavity. The spleens were plum-coloured with grey markings. The capsules were tense and the contents turgid. On incision the splenic pulp was semi-liquid and greyish red and the Malpighian corpuscles could not be distinguished. In two dogs, band infarcts were present.

In three dogs the livers were enlarged, the edges were rounded and extended beyond the costal arch. The organs were mottled and not unlike "nutmeg livers." One dog had sharply circumscribed liver lesions which were yellowish-grey in colour and measured about $\frac{3}{4}$ inch in diameter. Ascites was present in two of the three cases with liver involvement.

The mesenteric lymph nodes were enlarged in all five cases and one dog had a diffusely thickened duodenum. Macroscopically the lungs were involved in two cases the affected areas being firm and grey in colour. The remaining lung tissue was congested and oedematous. The adjacent lymph nodes were diffusely enlarged. Renal lesions in the form of cortical infarcts were present in three dogs. In all cases changes were present in the marrow which was softer than normal and had a mottled appearance. No macroscopic changes were noted in the central nervous system or other viscera not mentioned.

Histology. The microscopic changes can be summarised as comprising a diffuse hyperplasia of large round cells which were thought to be lymphoblasts. Many of them had a well-defined nuclear membrane and coarse chromatin. Neutral red stain did not indicate granules. A few of the cells had vesicular nuclei and were probably monocytic cells: they were present in very large numbers in the affected organs and tissues. In several dogs no normal architecture was detectable in the spleen and the organ comprised a mass of mononuclear cells. The lymph nodes shared this massive cellular infiltration and loss of structure. Some areas in certain nodes were necrotic; others showed haemorrhages and many free red cells. In no lymph node was there any evidence of fibrosis and no multinucleate cells were detected. Affected livers showed a periportal infiltration with lymphoblast cells and fatty changes in the hepatic cells. The lungs in two dogs contained many peribronchial and perivascular "cuffs" of mononuclear cells and the normal lymph follicles of the lungs were enlarged and hyperplastic. Histological examination of the marrow revealed a reduction in the number of fat spaces and an infiltration with the same type of cell as was present in spleen and lymph nodes. There was evidence of increased activity of the granular series of cells, but few red-cell regenerative changes were noted.

TREATMENT WITH URETHANE AND NITROGEN MUSTARD HYDROCHLORIDE

An opportunity arose to treat three of these cases with urethane. This was administered in gelatine capsules at a dose of 1g. daily. One dog was also given nitrogen mustard hydrochloride by the intravenous route.

Case No. 3, a Scotch terrier, was treated over a period of 19 days. The changes in the cell content of the peripheral blood may be seen in Table I. Clinically there appeared to be some reduction in the size of the superficial lymph nodes, the appetite was improved but the dog had bouts of diarrhoea. The animal died on the 20th day after it was first seen and came to post-mortem examination. The gross and microscopic lesions did not differ from those already described for the series.

Case No. 4 was treated with urethane for a period of 8 days. During this period the drug had no apparent effect either on the clinical course of the disease or upon the peripheral blood picture. The last blood sample was taken on the eighth day. The dog was not brought into the clinic again and although several attempts were made it was not found possible to contact the owners of the animal.

Case No. 8, a Scotch terrier, was given urethane daily for 20 days. The graph of the cellular reaction of the blood (Fig. 1) shows a slight initial fall in red cell count and the haemoglobin level

followed by a slight rise. These changes are not significant. The eosinophil count increased during the temporary neutropenia. On the 20th day of the urethane treatment this drug was replaced by nitrogen mustard hydrochloride given intravenously. Immediately after the injection the dog vomited and developed severe respiratory distress. The effect on the peripheral blood may be seen in Fig. 1. The blood changes were most marked on the 26th day when the red cell count had fallen to 2.9 million cells per cmm. and the haemoglobin value to 7.3g. per cent. There was a corresponding drop in the white cells and the total white cell count was 9.0 thousand cells per c.mm. On the 31st day the dog appeared much better: it was more alert, the appetite was good and the peripheral blood picture, except for the anaemia, was normal. Unfortunately, however, the owner refused to persist with further treatment and the dog was destroyed. The post-mortem findings indicated the widespread nature of the disease. All the lymphatic nodes were involved together with lesions in the lungs, liver, spleen, kidneys, duodenum and bone marrow. Sections of the organs showed rather more necrosis of the lymphoid elements than had been seen in other cases but whether this was due to the nitrogen mustard or to the advanced nature of the condition could not be determined.

DISCUSSION

It would appear from the literature that canine leukaemia is of the lymphogenous type. The gross and microscopical pathology of the visceral lesions appears to be the same in all cases (Wirth and Baumann, 1933; Engelbreth Holm, 1942; Hutyra, Marek and Manninger, 1938; White, 1946). The effect on the peripheral blood, however, may range from no change at all, through a leucocytosis, to a true lymphatic leukaemia. Such a wide variation in the blood picture is not perhaps surprising and an equally wide range may be seen in the leukaemic diseases of man. Engelbreth Holm's (1942) classification of the two main types of leukaemia would appear to be the most suitable. There was no evidence to suggest that the terms Hodgkin's disease or pseudo-Hodgkin's disease were in any way suitable. These terms merely add further confusion to an already over-classified disease. It would also appear from the literature that, to date, no authentic case of myeloid leukaemia has been recorded in the dog.

All the cases described in this communication would fall into the aleukaemic group except for Case No. 1 which could be classified as having a leucocytotic blood picture. No cases of lymphatic leukaemia with a leukaemic blood picture were encountered and this agrees with the findings of Bloom and Meyer (1945). It would appear that cases of lymphatic leukaemia with typical blood changes are the exception. The explanation may well be that the change in the blood picture occurs as a terminal phase in those animals which survive long enough. The leucocytic blood picture

is not difficult to understand when one considers the reaction which occurs as a result of the proliferation and infiltration of the lymphoblasts. The tissue damage and necrosis is quite sufficient to evoke a leucocyte response provided the formative tissues are not damaged.

The presence of an anaemia in this condition had been noted by previous workers, but generally it had been termed "a severe anaemia" or "a marked anaemia." In these 8 cases the anaemia has been classified. It is interesting that no cases of aplastic anaemia were encountered in canine leukaemia in the present series nor are they reported in the literature; the explanation is probably that the bone-marrow involvement was never sufficiently great to stop haemopoiesis. That the bone-marrow even in late stages of the disease was capable of regenerative changes was shown by the presence of normoblasts in the circulating blood.

In view of earlier reports in the literature and the findings in these 8 cases it is evident that examinations of the peripheral blood picture have little if any diagnostic or prognostic value.

The etiology of the condition requires little comment. There is still insufficient evidence to decide whether the condition is really neoplastic or if it is hyperplastic in nature. If it is the former it is quite impossible, on present evidence, to decide whether the lesions in the various organs arise by metastasis, or are examples of multifocal origin. The general impression from 8 cases was that the condition was a form of hyperplasia of lymphoid elements. There was nothing in the histological evidence to suggest that it was one of the reticuloses, and there was no resemblance to Hodgkin's disease as seen in man.

Innes *et al.* considered that urethane had a temporary beneficial effect. In two of the present series there was some slight clinical improvement, but one case died and the other one was destroyed so that no valid conclusions are possible. The drug is toxic and will eventually cause aplasia of the nucleated cells. It is doubtful whether it has a part to play in the treatment of this disease. The same may perhaps be said of nitrogen mustard which is even more toxic. It became obvious, even from the present very limited experience of these drugs, that their use must be governed by detailed clinical and haematological examinations.

SUMMARY

Eight cases of canine lymphadenosis are described and the literature is briefly reviewed.

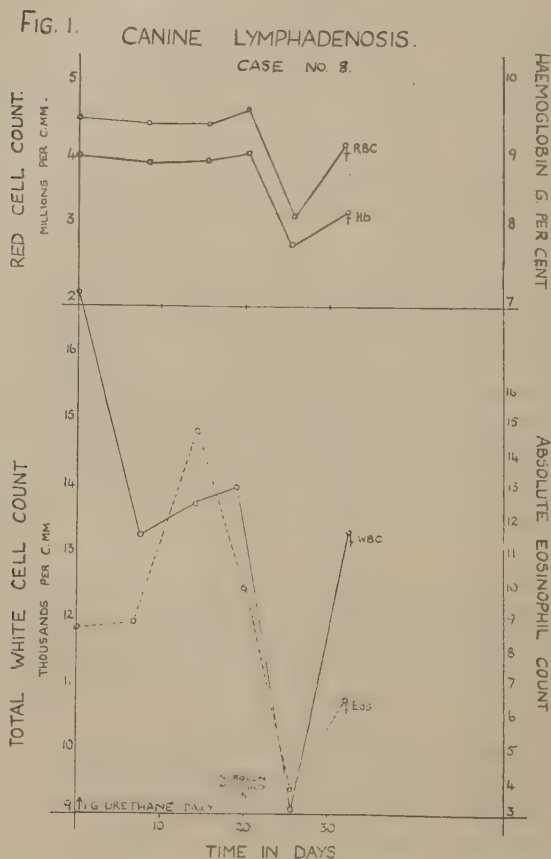
Mention is made of the treatment of the condition with urethane and nitrogen mustard.

REFERENCES

- Bloom, F., and Meyer, L. M. (1945). *Amer J. Path.*, **21**. 683.
Coffin, D. L. (1945). *Manual of Veterinary Clinical Pathology*, Comstock Publishing Co.; New York.

- Dahlström, H., and Henschen, F. (1918). *Svensk. veter. Tidskr.*, **23**, 496, 514, 548.
- Engelbreth-Holm, J. (1942). *Leukaemia in Animals*. Oliver and Boyd; Edinburgh.
- Holman, H. H. (1944). *Clinical Haematology in Boddie's Diagnostic Methods in Veterinary Medicine*. Oliver and Boyd; Edinburgh.
- Hutyra, F., Marek, J., and Manninger, R. (1938). *Special Pathology and Therapeutics of the Diseases of Domestic Animals*. 4th Edn. Bailliere, Tindall and Cox; London.
- Innes, J. R. M., Parry, H. B., and Berger, J. (1946). *Vet. J.*, **102**, 383.
- Jennings, A. R. (1952). *J. comp. Path.*, **62**, 161.
- Siedamgrotzky, O. (1871). *Ber. veterinärw. Sachsen.*, **16**, 64.
- White, E. G. (1946). *Proc. Roy. Soc. Med.*, **29**, 739.
- Wirth, D. (1931). *Grundlagen Einer Klinischen Hämatologie der Haustiere*. Urban and Schwarzenberg; Wien U. Berlin.
- Wirth, D. and Baumann, R. (1933). *Folia Haem.*, **50**, 242.

[Received for publication, August 19th, 1952]



LEPTOSPIROSIS IN CATTLE IN ISRAEL

By

J. VAN DER HOEDEN and CHEDVA HALEVY

The Veterinary Institute, Ministry of Agriculture, Tel-Aviv, Israel
and

I. DAFNI

District Veterinary Officer, Tel-Aviv.

INTRODUCTION

Until recently leptospirosis was mainly known as an infectious disease of man, dogs, pigs and wild rodents. Since Russian workers described outbreaks of leptospirosis in cattle (Michin and Azinow, 1935), the bovine disease has been observed in other countries, especially in Palestine (Freund, Trainin and Malkin, 1941), Australia (Johnson, 1942), the U.S.A. (Jungherr, 1944), the Argentine (Savino and Revella, 1944) and Japan (Yamamoto, 1951). In Australia and North-America *L. pomona* has been found to be the cause of the disease; in Japan *L. australis* and *L. hebdomadis* were identified serologically; in U.S.S.R. and Israel strains of *L. grippityphosa* were isolated. The detection of a large number of cattle in Switzerland with high serum agglutinins mainly against *L. sejro* but also against other types of leptospira (Wikerhauser, 1951), indicates that bovine infections also occur in areas where clinical cases have not yet been observed in these animals. Similar results were obtained in Denmark by Petersen (1944) (*L. sejro* and *L. saxkoebing*), in Bavaria by Rimpau (1950) (*L. grippityphosa* and *L. sejro*), in Silesia by Kathe (1943) (*L. sejro*, *L. grippityphosa* and *L. australis* A) and in Italy by Babudieri (1949) (*L. icterohaemorrhagiae* and *L. pomona*).

The present paper gives a description of a sudden outbreak of leptospirosis in a herd of cattle in a communal agricultural settlement (Shefayim) in the coastal plain of Sharon in March, 1949.

COURSE OF THE ENZOOTIC AT SHEFAYIM

Three samples of blood were sent to the Veterinary Institute at Tel Aviv, for serological examination on 2.3.1949. One sample (cow) revealed an agglutination-titer of 1:200 with the leptospiral strain of bovine origin which had been isolated by Bernkopf (1946) from a calf in this country in 1945, as well as with *L. grippityphosa* (strain Moskou V) to which it is narrowly related: the others were negative. It was then decided to examine sera of all the cattle present in the settlement and the results are summarized in table I.

More clinical cases occurred and in order to follow the development of the enzootic, blood was collected again on 8.5.49 from adult and young cattle which at first examination had not reacted. The number of calves had then increased from 7 to 22. In addition the blood-sera of some of the horses were tested. The results are given in table II.

TABLE I

Agglutination-titer with <i>L. grippotyphosa</i>	Shed I 22.3.49	Shed II 15.3.49	Shed III 15.3.49	Calves 22.3.49	Total number of reactions
0	1	16	8	6	31
1:20	10	9	3	0	22
1:200	8	2	2	0	12
1:2000	8	3	2	0	13
1:20,000	17	0	0	1	18
1:200,000	6	0	0	0	6
Titer 1:200 or higher	39	5	4	1	49
Total number of animals examined	50	30	15	7	102

TABLE II

Agglutination-titer with <i>L. gr. typhosa</i>	Shed I	Shed II	Shed III	Calves	Equines
0	0	7	0	8	6
1:20	1	3	0	2	2
1:200	0	2	1	4	1
1:2000	0	1	1	0	0
1:20,000	0	3	6	7	0

Of the 25 adult cattle which had not reacted to the first test 18 gave a positive reaction to the second. However, only one of the 6 previously negative reacting calves now showed the presence of agglutinins in a concentration of 1:200. Taking the two tests together 12 of the 22 calves had a significant serum-reaction (1:200 or above) and two gave a slight reaction (1:20).

The infection had thus spread rapidly amongst the cattle so that two months after the first cases were observed, 88 out of all 95 adult cows and 14 out of 22 calves had produced specific agglutinins for *L. grippotyphosa* and the bovine local strain. Finally 113 adult and young cattle which made up the herd on 20.11.49 were examined. Of 84 animals that were tested previously, five which had never shown agglutinins before, were still negative; in 22 cases there was no change; there was a fall in titer in 51 and a rise in 6.

Clinical Course.

The first clinical cases were observed on 2.3.49 and the last one on 31.5.49. Altogether 12 animals, all adults, were visibly affected as compared with more than one hundred that reacted to the agglutination test. Of the 12 sick cows, six were in shed II where 30 beasts were housed and six in shed III where 15 animals were present. In shed I, all but one of the 50 animals gave positive agglutination reactions on 22.3.49 (see table I); nevertheless, no sick cattle were observed. In response to questioning, however, the cowmen recollected that for some time past, individual cattle in that shed had refused their food for a day or two and also shown a rise in

temperature. There were also a number of unexpected abortions among heifers. The herd, which is under control, was regarded as free from brucellosis, moreover all aborting animals proved negative to the serum-agglutination test for brucellosis. As to the calves, housed in several pens, no clinical symptoms were observed although, as stated before, twelve showed a positive serum-reaction of 1:200 or over. The symptoms manifested by the adult cattle were similar to those originally described by Freund, Trainin and Malkin (1941) in cases of icterohaemoglobinuria in bovines in Israel. They showed a marked rise of temperature up to 40°C to 41°C, lasting one or two days, followed by an orange coloured icterus of the mucous membranes which was more marked in some than in others. In a number the milk and urine became bloodstained. All the animals rapidly became emaciated and the milk yield was reduced almost to nothing. In two cases the course of the disease was very acute, leading to death or emergency slaughter within a week. Two showed a sub-acute course, the symptoms gradually disappearing in about two months from the onset of the disease and the milk returning to about 80 per cent. of its original yield. In one case the disease was prolonged so that ten weeks after onset the animal was still thin and completely dry, necessitating its slaughter. The remaining seven cows had a relapse 5 to 8 weeks after the onset of the disease when it seemed that they were on the way to recovery: within a week, two of these animals died and the remaining five were slaughtered *in extremis*.

The following is a detailed description of the clinical picture and laboratory findings of nine prominent cases:—

Case 1 (5 years old) was the first clinical case observed. The disease started with a high temperature for one day. The animal stopped feeding, had orange mucous membranes and rapidly became emaciated. The loss of milk was almost complete. In about six weeks after the acute symptoms had subsided, the animal was emaciated and did not return to milk. The post-mortem examination showed a very intense interstitial nephritis. In the early stages the agglutination titer of the blood was 1:200. Thirteen days later it had risen to 1:20,000. The titer of the urine never surpassed 1:100. *Leptospira* were detected in the urine by dark ground examination. The whey agglutinated *L. grippityphosa* to a dilution 1:20,000 on several occasions.

Case 2 (4 years old) was a relatively mild case which gradually recovered and in about two months returned to 80 per cent. of her original milk yield. No agglutinins for leptospira were detected in the blood on 2.3.49 and 15.3.49. On 8.5.49 the blood titer and the milk titer were both 1:20,000. On 20.11.49 the blood titer was still 1:10,000 and one year later it was 1:200.

Case 3 (9 years old) became ill on the same day as the former two cows, showing similar clinical symptoms. She made a partial recovery, but then relapsed and died within a few days. The blood titer which was negative on 2.3.49 became 1:20,000 on 15.3.49 when the milk titer was 1:2,000 and the urine titer 1:20.

Case 4 (2½ years old) showed the first symptoms on 24.3.49 and suffered from a severe relapse about 8 weeks after onset of the disease. On the eleventh day of illness the serum had already attained an agglutinin titer 1:20,000. At this date the blood picture demonstrated the following pathologic changes: leucocytosis, anisocytosis, poikilocytosis, normoblasts and basophilic punctation of erythrocytes. On 8.5.49 the agglutination titer of the milk was 1:20,000 but that of the urine was only 1:50.

Case 5 (8 years old) showed the first symptoms of disease on 19.4.49. The animal partly recovered but suffered a relapse at the end of May showing marked jaundice, emaciation and loss of milk and was slaughtered. On 15.3.49 no agglutinins were encountered in the blood. Three days after the appearance of the first clinical symptoms, the blood titer reached 1:20,000. On 8.5.49 the milk titer also rose to 1:20,000, the urine titer being 1:20.

Case 6 (4 years old). On 25.4.49, the second day of illness, no agglutinins were found in blood and milk. The first positive blood serum reaction (1:2000) was noticed on 8.5.49 when the milk titer was 1:200, but the urine failed to show any agglutinins. On 20.11.49 the blood still agglutinated *L. grippotyphosa* in a dilution of 1:1000. The animal recovered gradually.

Case 7 (5 years old) suffered a relapse about five weeks after the first attack. In the first stage of the disease the blood titer was 1:20,000, but the milk titer only 1:20. The animal was slaughtered on account of loss in condition. *Leptospira*, belonging to the grippotyphosa-group were cultivated from the kidneys. Agglutinin-absorption tests revealed an identity with the strain originally isolated by Bernkopf. Sections of the enlarged kidneys showed an extensive interstitial nephritis and many leptospira were seen in preparations stained by the Levaditi method.

Case 8 (4 years old) died six weeks after the onset of symptoms. No agglutinins were found in the blood serum. This acute case was characterized by loss of appetite, high fever, appearance of blood in milk and urine, total arrest of milk secretion and intense jaundice. The post-mortem examination revealed congestion of the internal organs, intense icterus of the fat and serous membranes as well as the membranous capsules of the muscles. The kidneys were enlarged and showed a very severe acute interstitial nephritis. A great number of leptospira were seen in sections of the kidney.

Case 9 (3½ years old) showed fever for one day only. The disease was as acute as Case 8. She was slaughtered on the sixth day of illness. The blood titer was negative on 15.3.49 and 1:200 on 1.6.49. At the time of slaughter it had risen to 1:1000, while the milk titer was 1:200. At post-mortem examination there was an interstitial nephritis and a great number of leptospira were found in the kidney. *Leptospira* of the grippotyphosa type, serologically identical with the strain of Bernkopf, were cultivated from the kidneys.

LEPTOSPIROSIS OUTSIDE SHEFAYIM

No clinical cases of leptospirosis were observed in bovine herds in the vicinity to Shefayim. In addition, blood samples collected on a number of occasions and submitted to the agglutination test with

L. grippotyphosa were mainly negative although about 5 per cent. gave reactions in a dilution 1/200 or above (table III).

TABLE III

Month 1949	Place	Agglutination titers		Remarks
		0-1/200	1/200 or above	
May	Rishpon (1 shed)	6	2	Owner sick with leptospirosis.
"	Givat-Hen (2 sheds)	16	0	Owners sick with leptospirosis.
"	Herzliya	197	14	
"	Herzliya B.	30	3	
"	Ramat Hasharon	67	1	
"	Kefar-Saba	173	11	
July	Kibutz Aleumim (Herzliya)	23	0	
August	Kibutz Eyal (Kefar-Saba)	10	0	
"	Gil-Yam	51	3	
November	Rishpon	60	4	
"	Kefar Shemaryahu	86	3	
"	Givat-Hen	110	2	
Total		829	43	

In more remote districts only a few sporadic cases of leptospirosis in animals were encountered during 1949.

HUMAN CASES OF LEPTOSPIROSIS

In view of former experiences in this country concerning the pathogenicity of bovine leptospirosis for man, the blood sera of persons working in close contact with the infected cattle were examined. On 28.3.49 none of the 13 sera from the attendants showed the presence of leptospira antibodies. Several months elapsed after the first animal case was observed before human cases appeared in Shefayim. According to information from the local physician, Dr. W. Cohen, the total number of persons showing clinical symptoms was twelve. With the exception of one man who acted as butcher and another who worked on the farm none of them were in direct contact with the infected animals. Blood from four of the sick persons was examined at the Veterinary Institute in August, 1949. Two gave a positive reaction with *L. grippotyphosa* and not with *L. icterohaemorrhagiae* or *L. canicola*. One of the patients belonged to the group of 13 persons with negative sera mentioned above. It is noteworthy that a considerable number of human cases of leptospirosis were diagnosed by the health authorities, between July and December, 1949, in several settlements mentioned in table III, in the vicinity of Shefayim, where no clinical cases occurred in cattle. At Shefayim 12 out of a population of 700 settlers including 300 children suffered from leptospirosis while in the same period during 1949 in the near-by settlement of Rishpon, with 380 inhabitants including 120 children, Dr. Cohen reported that no less than 47

human patients contracted the disease. Clinical cases of infection in cattle were not encountered at Rishpon.

EXAMINATION OF RODENTS

Olejnik and Shneyerson (1950) came to the conclusion that during the human epidemic of 1949 to 1950, an epidemiological relationship existed between infection of voles with leptospira and leptospirosis in man. Furthermore they stated that a new strain, related to but not identical with *L. grippotyphosa* and *L. bovis* had been transmitted by *Microtus guentheri* in the field.

The presence of agglutinins for *L. grippotyphosa* in the blood sera of *Microtus guentheri* had already been detected by the Veterinary Institute in voles which were caught in several places in the coastal plain of the country where cases of leptospirosis had occurred among the population or where reacting cattle were present. Moreover Dr. Zlotnik detected leptospira in Levaditi-stained sections of the kidneys of those voles whose blood sera showed high agglutination titres for *L. grippotyphosa*, whereas no leptospira were detected in kidneys of non-reacting microtus.

The large numbers of rodents which were present during the period when the enzootic occurred at Shefayim lent strength to the assumption that these animals might be the vectors of the leptospira and therefore responsible for the outbreak amongst the cattle.

From 22.12.49 onward, rats, voles and mice were caught in Shefayim and its vicinity. Their blood was tested for agglutinins and the organs were examined for leptospira by dark ground illumination and cultural methods. One, out of 73 rats (*Mus. alexandrinus*) examined showed a positive serum agglutination titer of 1:1000 with *L. grippotyphosa* and a negative reaction with *L. icterohaemorrhagiae*. No agglutinins were found in the blood of a house-mouse (*M. musculus*) and eight Merinoes. On 21.2.50 the blood sera of eight voles (*Microtus guentheri*) were examined. Two were positive, one with an agglutinin titer for *L. grippotyphosa* of 1:100, and the other of 1:1000. No leptospira were recovered from the kidneys by cultural methods. Sections of the kidneys of the last mentioned vole showed interstitial nephritis and leptospira within the tissue. In voles with negative sera, leptospira were not found. The positive voles had been trapped at some distance from the sheds, but the cows had to pass the spot on their way to pasture.

DISCUSSION

In the outbreak reported in this paper only a small proportion of cattle showing significant agglutination titers for leptospira were affected with clear-cut symptoms of disease. The majority probably suffered from a subclinical attack which passed unrecognized.

It is of interest that all the clinical cases appeared in sheds II and III and none in shed I. In this connection it is noteworthy that at the beginning of the enzootic 39 out of the 50 cows in shed I (approx.

80 per cent.) showed significant agglutinin-titers, whereas only 9 of the 45 cows (20 per cent.) in sheds II and III were positive. It is postulated that a resistance to leptospira infection had developed in the cattle in shed I at an earlier date than in the cattle in the two other sheds. If the virulence of the leptospira increased during the course of the enzootic, the cattle in shed I may have already acquired sufficient immunity to prevent the appearance of clinical cases. Furthermore nearly all the cows in sheds II and III which became affected showed no agglutinins at their first blood examination.

The symptoms of disease varied. Whilst fever, jaundice, emaciation and loss of milk secretion were constant, blood staining of milk and urine appeared only in a certain number of cattle.

There was a great variation in the severity of symptoms and the course of illness. Most of the cases made a partial recovery, then relapsed and died or had to be slaughtered. One animal died and another was slaughtered *in extremis*, within a week from onset of symptoms. Only two animals gradually recovered.

Whilst it is clear that leptospirosis encountered in man and in cattle in the Shefayim area was caused by the same type of organism, there is much evidence to support the contention of Olejnik and Shneverson (1950) that the infection of man was not derived from cattle but probably from voles. The first human cases started at a time when the disease in cattle was already subsiding. Only one or two of the patients were in close contact with cattle: all the others worked in the vegetable gardens where an extraordinarily large number of rodents were present. The pepperplants which were handled by the workers were especially damaged by voles. It is also obvious that the incidence of human leptospirosis in several other settlements mentioned above did not coincide with the disease in cattle. There were areas where many human cases occurred with very few or no bovine cases and vice versa. All these facts throw doubt on the former belief that human leptospirosis was contracted from sick cattle, and strengthen the assumption that voles might be vectors of leptospirosis both in man and animals in this country.

In a publication by one of us (van der Hoeden, 1951), the following assumption was made. In certain periods as in 1946 to 1948, the number of rodents present was relatively small. They caused only sporadic infections in cattle while man, who had little contact with field rodents, was still less likely to become infected.

During such a period the sporadic human cases which arise are probably due to contact with infected cattle. When as happened in 1949 to 1950 the number of rodents shows a substantial increase, the fields become heavily contaminated with their excretions, and the risk of infection amongst field workers is correspondingly increased.

In normal periods the disease tends to attack butchers, veterinarians and cattle breeders, whereas in times of abnormal multiplication of voles, agricultural workers in the field are more likely to become affected.

SUMMARY

A description is given of a severe outbreak of bovine leptospirosis during the course of which 12 adult animals showed marked clinical symptoms.

Two animals only recovered: the remainder died during the acute phase or having made a partial recovery, relapsed and died or were killed.

Three weeks after the onset of the disease the sera of 49 out of 102 cattle agglutinated *Leptospira grippotyphosa* at a dilution of 1:200 or over. High agglutinin titers were also encountered in the milk.

At post-mortem examination there was advanced interstitial nephritis and the organism was usually demonstrated in sections or by cultural methods.

Cases of leptospiral infection in man occurred when the bovine disease was subsiding. As two out of eight voles (*Microtus guentheri*) were infected and rodents were abundant in the area, it is supposed that they were the main reservoir of the agent transmitting infection to both cattle and man.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. Zlotnik, former head of the Section of Histopathology of the Veterinary Institute for the histological examination mentioned above. They are also indebted to Miss H. Lubecki who undertook a part of the serologic examinations in the Section of Bacterial Diseases of this Institute, as well as to Dr. Dreyfuss, the local veterinarian and to Dr. W. Cohen, physician of Shefayim, both of whom were most helpful in providing valuable information.

REFERENCES

- Babudieri, B. (1949). *Rend. Inst. sup. Sanita*, **12**, 937.
Bernkopf, H. (1946). *Harefuah*, **30**, 110.
Freund, Trainin, S. D. and Malkin, M. (1941). *The Palest. vet. Bull.* **8**, 153.
Hoeden, J. van der (1951). *Bull. Off. int. Epizooties*, **36**, 400.
Johnson, D. W. (1942). *Med. J. Austral.*, 431.
Jungheer, E. (1944). *J. Amer. vet. med. Ass.*, **105**, 276.
Kathe, J. (1943). *Z. Immun Forsch.*, **103**, 60.
Michin, N. A. and Azinow, S. A. (1935). *Sovyet Vet.*, **10**, 23.
Olejnuk, E. and Shneyerson, S. (1950). *Nature*, **166**, 526.
Petersen, C. Borg (1944). *Acta path. Microb. scand.*, **21**, 156.
Rimpau, W. (1950). *Die Leptospirose*, Munchen, Urban and Schwarzenberg.
Savino, E. and Revella, E. (1944). *Rev. Soc. Argent. Biol.*, 20.
Wikerhauser, T. (1951). Thesis Berne.
Yamumoto, (1951). *Bull. Off. int. Epizooties*, **36**, 421.

(Received for publication, September 18th, 1952)

LEPTOSPIROSIS AMONG GOATS IN ISRAEL

By

J. VAN DER HOEDEN

Veterinary Institute, Ministry of Agriculture, Tel-Aviv, Israel.

INTRODUCTION

When Freund, Trainin and Malkin (1941) first recorded icterohaemoglobinuria in cattle of Jewish settlers in Palestine, a similar condition, affecting native cattle as well as sheep and goats, was already known to the local Arabs under the name of "safra". The special attention of the Government Veterinary Service of Israel was attracted when jaundice in epizootic form occurred in several Arab villages of the Haifa district during the year 1949. The following is a quotation from an official report to the Director of Veterinary Services.

"Though the exact number of fatalities is unknown it is a matter of fact that the damage in goats, caused by this disease, is considerable. Out of a herd of 120 goats in Shefar-Am, 17 died and only 4 or 5 recovered from the disease, all of them showing intensive jaundice. In Churfesh more than half of the goats died in February, 1949. Similar observations were made in Gush-Halav and in a few other Arab villages of the Haifa- and Safad-districts. The most important symptoms of the disease were lack of appetite and jaundice; many animals aborted."

The results of laboratory examinations carried out in the Veterinary Institute at Tel-Aviv, supported the supposition that leptospira had been a causative factor, but it was impossible to determine exactly the part played by this micro-organism since ecthyma, scabies, pox, helminthiasis and anaplasmosis were also present in the affected herds.

In February 1949, three out of 13 sera from sick goats of Beer-Am agglutinated *L. grippotyphosa* in dilutions of 1:200, 1:2000 and 1:10,000 respectively. Two goats from a neighbouring place showed titres of 1:200 and 1:20,000 respectively in March. From the village of Shefar-Am one goat reacted at 1:200 and one at 1:2000 while during May, 2 goats showed titres of 1:2000 and 5 of 1:20,000. Out of ten jaundiced goats, three from Shefar-Am and one from Churfesh showed the presence of leptospira in sections stained by Levaditi's method (Dr. I. Zlotnik).

Similar outbreaks in goats have most probably occurred in Palestine in the past. According to du Toit the extraordinarily high mortality of 32 per cent. in Palestinian goats, mentioned by Smith as due to anaplasmosis (Hutyra, Marek and Manninger, 1949), may have been caused by other agents and our results, which will be described below, support du Toit's suggestion. It is known that the carrier-rate of anaplasmosis in native goats in this country is high whereas losses from the disease itself are very slight. Therefore it is

logical to suppose that heavy losses are caused by some other agent and our investigations make it probable that leptospira, which may give rise to a clinical picture resembling anaplasmosis, plays an important role.

HISTORY AND SYMPTOMS

In November, 1951, a sick goat from the Arab village of Meissar was delivered to the Veterinary Institute and died as soon as it arrived there. It had aborted the day before and was severely jaundiced. During the previous six weeks several goats had been similarly affected, the general symptoms being lack of appetite, intensive jaundice and excretion of dark reddish or brown urine during two to four days. Many animals had aborted. Originally the disease was considered to be anaplasmosis but later on leptospirosis was suspected. Inhabitants of the village reported that two icteric goats had been slaughtered and their meat eaten without causing any harm to the consumers. A similar case was reported from a cow.

Post-mortem and Histological Examination

The carcass was emaciated and all tissues were intensely yellow. The lungs showed marked lesions of verminous bronchopneumonia, and petechiae were present on the visceral pleura. The lymph nodes were congested and oedematous. The kidneys showed venous congestion, degeneration and haemorrhages in the medulla, with swelling, necrobiosis and desquamation of the epithelial cells of the tubules and the presence of hyaline and cellular casts. There was some evidence of interstitial nephritis with mononuclear infiltration especially of the perivascular and periglomerular areas. The glomeruli were swollen, their capillaries empty and the visceral and parietal layers of Bowman's capsule slightly proliferated. In several glomeruli the capillary tufts filled the capsular spaces; in others the spaces contained eosinophilic droplets. The liver showed venous congestion with degeneration and necrobiosis of the liver cells, most marked around the central veins. The Küpffer's cells were swollen. Slight infiltration with mononuclear cells and lymphocytes was present in the portal spaces, especially around the bile-ducts. The Malpighian bodies of the spleen were small and widely separated and the sinuses of the red pulp congested and dilated, particularly around the lymphatic nodules. Large numbers of macrophages, some containing erythrocytes were present in the red pulp. The large intestine was congested. The urinary bladder contained a darkish-brown fluid. In Levaditi-stained sections of the liver and kidney many leptospira were seen.

Serological Examination.

The blood-serum agglutinated *L. grippotyphosa* (strain "Moscou V" and the local strain "calf-Bernkopf") up to a dilution 1:3000. The agglutination titer of the milk whey and of the urine was 1:80. No

agglutinins were found against *L. icterohaemorrhagiae*, *L. canicola*, *L. pomona*, *L. hebdomadis*, *L. sejroe* and *L. mitis* (Johnson).

Isolation of Leptospira.

Direct cultural examination in Korthof's medium of liver, kidney, heart blood, milk taken from the udder, and urine from the urinary bladder was successful in the case of the first two organs only. *Leptospira* were, however, recovered from each of the other materials by culturing from the liver and kidneys of white mice, guinea-pigs and hamsters which had been inoculated intraperitoneally with the samples.

Antigenic Structure.

Sera of rabbits, immunized with different strains of *L. grippotyphosa* (Moscou V, strains isolated from man and cattle in Israel) agglutinated the goat strain to the same titer as the homologous strains. No, or very slight, agglutination was seen in sera prepared against *L. icterohaemorrhagiae*, *L. canicola*, *L. pomona*, *L. hebdomadis*, *L. sejroe*, *L. saxkoebing*, and *L. ballum*. The serum of a rabbit immunized against the goat strain agglutinated the other *grippotyphosa* strains to practically the same dilution as the homologous Meissar strain. No antigenic relationship, however, was shown with the other leptospira types. In agglutinin-absorption tests the Meissar strain proved to be identical with *grippotyphosa* strains formerly isolated from cattle in this country.

INVESTIGATIONS IN ARAB VILLAGES

Village Meissar.

Three herds were examined in November, 1951.

Herd 1 comprised 120 goats, 120 sheep and 20 cattle. On that date 14 goats were reported as having died previously, three were still sick and one had partly recovered. The symptoms of the disease were described as lack of appetite of two to four days duration, icterus and the passage of dark brown or red urine. No definite rise of temperature was noted in twenty sick animals as compared with apparently healthy goats.

In one goat which had aborted and was still slightly icteric, the blood-serum agglutinated *L. grippotyphosa* to 1:3000 and the milk whey to 1:80. A second goat showed very intense jaundice of the mucous membranes and the urine was brown. On the fourth day of illness no agglutinins were present, but thirteen days later the blood-titer had risen to 1:10,000; the milk titer 1:2500. A third goat had very marked jaundice and as early as the fourth day of illness the agglutinin titer of the blood was 1:30,000.

No symptoms of illness were noted among the sheep and cattle although they were constantly in close contact with the goats.

Herd 2 comprised 46 goats of which four had died during the previous weeks and four were still ill. One goat showed a very marked jaundice and haemoglobinuria. The serum titer for *L.*

grippotyphosa was 1:1000. A second goat suffered a very severe attack of illness but subsequently improved. The agglutinin titer was 1:3000.

Herd 3 comprised 77 goats and 67 sheep. Five goats had died and three were still suffering from the disease. All the sheep had remained healthy except a ram which was off its appetite for two days. The ram's serum agglutinated *L. grippotyphosa* to a dilution of 1:30,000, and the titre was unchanged 14 days later. In one goat which had aborted and was showing slightly yellow mucous membranes, the agglutinin titer of the blood was 1:1000; that of the milk was 1:20. In a second which had almost recovered from a severe attack of illness with icterus, the serum titer was 1:3000. A third goat had been ill for seven days, had aborted and was very weak. The titre of the serum was 1:3000, and that of the whey was 1:20.

It was ascertained that up to December 6th, out of 249 goats, 50 had died, nearly all of them showing marked jaundice and blood-stained urine; many of them had aborted. Forty out of 43 clinically-affected goats in the above mentioned three herds died. No definite symptoms of disease were observed among the other species of animals (cattle, sheep, horses, donkeys, dogs).

Blood sera of the three shepherds were examined in November. The serum of a boy of 14 years (herd 1), agglutinated *L. grippotyphosa* to a dilution of 1:1000 but did not react with *L. icterohaemorrhagiae* or *L. canicola*. The boy said he had not been ill during the past year. The two other shepherds, aged 23 and 26 respectively, possessed no agglutinins.

The sera of four out of six goats which had shown no symptoms of disease agglutinated *L. grippotyphosa* to a high titre (1:3000) as also did one of five clinically healthy sheep. Out of seven "normal" cows one serum reacted in a dilution 1:10, one in dilution 1:30, one in a dilution 1:300, and one in a dilution 1:3000.

These findings led to an investigation of the existence of agglutinins in all the domestic animals of the village with the results shown in table I.

TABLE I

Species	No. of sera examined	Negative		Agglutination-titres with <i>L. grippotyphosa</i>					1:300 or above
			10-30	100	300	1000	3000	or more	
Goats	115	32	10	10	3	17	43		54.8 per cent.
Cattle	131	36	11	14	14	25	31		53.4 per cent.
Sheep	34	18	1	2	2	7	4		38.2 per cent.
Horses	5	1	—	—	1	1	2		
Donkeys	12	6	—	2	—	2	2		
Dogs	6	2	—	—	—	2	2		

Assuming that a titer 1:300 is significant, the collected data show that more than half of the goats and the cattle had been infected with *L. grippotyphosa* and that many infections had also occurred among

sheep, horses, donkeys and dogs. The choice of 1:300 as the critical titer indicating infection was based on the results of a large number of tests of blood from several species of domestic animals and man in other areas of the country which showed that agglutination of *L. grippotyphosa* to a dilution of 1:100 was exceptional.

The real extent of infection in herds can be revealed only by testing the samples of serum on a number of occasions so as to detect rises in titer, for it is evident that only a proportion of the animals which become infected develop symptoms of the disease. Unfortunately, circumstances were such that the human population of the village could not be examined. The agglutination titer of 1:1000, in one of the three shepherds, however, may be considered as indicative of past infection. It is remarkable that, with rare exceptions, goats were the only animals to show definite symptoms of disease and that the few cases seen among sheep and cattle ran an atypical course which ended in early recovery. It must also be borne in mind that only a minority of the goats which become infected showed definite symptoms of disease.

Positive serum reactions in dogs were observed with *L. grippotyphosa* only; no agglutinins against *L. ictero-haemorrhagiae* or *L. canicola* were found. Three of the dogs from Meissar were killed for further examination. One of them had a titer of 1:2000, another 1:4000 and the third showed no reaction. *Leptospira* were not found in Levaditi-stained sections of the kidneys of the three dogs. No growth appeared in culture-media and injection of kidney suspensions into guinea-pigs, hamsters and white mice gave a negative result. All three dogs, however, showed tubular degeneration in the kidneys, and the one with the highest titer had a slight interstitial nephritis.

The number of sick goats showing marked jaundice and haemoglobinuria was twenty-two. At the same time a calf which had almost recovered from slight disturbances (lack of appetite, sub-icteric mucous membranes) showed a serum agglutinin titer with *L. grippotyphosa* of 1:3000. Six out of fifty-one sheep showed slight dullness but all had recovered within a week by which time two of them had a titer of 1:100 and a third of 1:1000. Two sheep which had previously recovered from a mild attack, reacted at 1:1000 and 1:3000 respectively. Information was elicited that in the village one adult sheep had died in November 1951 and fourteen lambs in January 1952, but in none of these cases was material sent for examination.

No fatalities occurred among 157 cattle, 9 horses, 22 donkeys and 8 dogs present in the village and no cases of illness were observed among the human inhabitants although they were in very close contact with the infected animals. On the other hand, between November 1951 and February 1952, 106 of the 240 goats of Meissar died (44 per cent. mortality) and almost all of these fatal cases had shown icterus and haemoglobinuria. Of the goats which presented

TABLE II.

Herd	Species	Date (1952)	No. of animals ill with jaundic.	No. of animals examined	Agglutinin titres with <i>L. grippityphosa</i>											
					Blood					No. of animals						
					0	20	1,000	2,000	10,000	20,000	animals	0	20	40	200	4000
No. 1	goats	18.2	0	84	82	2					21	21				
"	"	19.2	1	1	1						1	1				
"	"	19.2	1	1				1			1		1			
"	"	21.2		same						1						1
"	"	19.2	0	16	9	2			5		10	6	1		3	
"	"	24.2	2(a)													
"	"	24.2	2	2						1						
"	"	24.2	0	23	9	5	4	5								
Other herds	"	19.2	1(b)	1						1						
"	"	19.2	0	40	16	17	5			2						
"	"	6.6		1(c)				1								
"	"	6.6		1(d)				1			1					
"	"	6.6		2(e)	2						1		2	2		
No. 1	cattle	19.2	0	5	5											
"	donkeys	19.2	0	2	2											
"	sheep	19.2	0	6	2	1	1		1							
Other herds	"	24.2	0	50	45	2		1	2							
"	"	6.6		1(f)												

- (a) died after one day.
 (b) recovered after three days illness.
 (c) a month after recovery.
 (d) two months after recovery.
 (e) recovered.
 (f) a week after recovery.

characteristic symptoms of leptospirosis only about 20 survived to make a gradual recovery. Most of the sick ones which were pregnant, aborted.

The last sick goat was observed at the beginning of April 1952. In May, blood was collected from 23 goats which were already adults at the time when the epizootic developed and from 17 goats which had been born after the disease had subsided. Only three of the last group of young animals showed significant agglutinin titers, whereas 19 of the old goats gave positive reactions. In view of these findings it is reasonable to suppose that the epizootic subsided when it did because almost all surviving animals had gradually contracted a certain amount of immunity against the leptospira. The decline may also have been due in part to the change from wet winter weather to the dry sunny season.

Village Baka

In the neighbouring village of Baka a goat died in January 1952 showing intense jaundice which had started a day before. Table II gives a summary of the data concerning the examination made in the herd to which this animal belonged and a few other herds in Baka.

In July, 1952, it was ascertained that during the previous two months seven goats had died in herd No. 1 and four goats and a sheep had recovered; all these animals had shown jaundice. In the other herds eighteen goats had died; seven goats and one sheep had been affected but had recovered.

Village Um-Al-Kutub

In the neighbouring Arab village of Um-Al-Kutub, the situation on 24.1.1952 was that ten goats had died and nineteen were still ill. Details are given in table III.

TABLE III

Herd No.	No. of goats	Died	Ill	No. of sera examined	Serum agglutination tests with <i>L. grippotyphosa</i>				
					0	20	100	1000	10,000
I	51	3	7	15 (6 ill)	15	—	—	—	—
II	42	5	7	20 (7 ill)	17	—	1	1	1
III	41	1	4	14 (4 ill)	13	1	—	—	—
IV	27	1	1	5 (1 ill)	5	—	—	—	—
Total	161	10	19	54 (18 ill)	50	1	1	1	1

On 19.2.1952 sera of nine clinically healthy cows were examined. Two gave negative reactions, two had a titer of 1:200, four of 1:2000 and one of 1:20,000.

Village Ain-Al-Saalah.

The data noted are summarised in Table IV.

TABLE IV

Species	No. of animals	Died	Ill	No. of sera examined	Serum agglutination test with <i>L. grippotyphosa</i>			
					0	20	200	1000
Goats	300	4	9*	52	33	16	2	1
Cattle	39	0	0					
Donkeys	15	0	0					

* Three of these goats died during the following week.

Village Bartaa

The situation in the village of Bartaa is summarised in Table V.

TABLE V

Species	No. of animals	Died	Ill	No. of sera examined	Serum agglutination test with <i>L. grippotyphosa</i>			
					0	20	200	1000
Goats	336	13	4	31	20	11	0	0
Cattle	10	0	0	4	2	0	0	2
Donkeys	2	5	1	0				
Horses	1	0	1	1	1			

The horse had been ill for one week and was jaundiced. The cause of its illness and that of the donkey could not be ascertained.

Up to the end of February, 1952, no additional cases had occurred at Bartaa but it was stated that the disease was still prevalent in the southern part of the village and along the border belonging to Jordan State.

In the Jewish settlement of Ain-Shemer, closest to the Arab area where the enzootic occurred, no goats but a considerable number of imported breeds of sheep were present. No symptoms suggestive of leptospirosis had been noticed in the flock. Twenty-four blood sera and twelve milk samples of these sheep were tested for agglutinins in February, 1952. The results were negative.

PATHOGENICITY OF THE LEPTOSPIRA STRAIN.

The Meissar strain proved to be pathogenic by intraperitoneal and subcutaneous injection for white mice, white rats, guinea-pigs, hamsters and several free living rodents in Israel (*Meriones shawi*, *M. crassus crassus*, *M. crassus sacramentus*, *Gerbillus pyramidum* and *Microtus guentheri*). In all these animals interstitial nephritis was caused.

Hamsters. Five out of eight hamsters died from leptospirosis in from 11 to 22 days after they were injected with milk, urine, and liver and kidney material collected from a goat at post-mortem examination. Three out of four died 10 to 13 days after injection of a culture of this strain, the fourth having been killed after seven days. Six out of 22 infected hamsters showed more or less intense jaundice.

Guinea-pigs. *Leptospira* were isolated from the blood of a guinea-pig eight days after infection; they were also recovered from the kidneys of four out of eight guinea-pigs at intervals of up to 34 days after intraperitoneal inoculation of material from the goat or a culture of the strain. Jaundice was not observed.

White mice. *Leptospira* were cultivated from the liver and kidneys of white mice which had been injected under the skin or into the abdominal cavity. Dark-field examination showed that one mouse excreted *Leptospira* with the urine up to 140 days, and the organism was cultivated from its kidneys when it was killed 190 days, after it had been infected intraperitoneally. None of the 22 infected mice showed icterus.

Field rodents. *Meriones* and *Gerbillus* died within four to eight days after the culture was introduced subcutaneously. *Gerbillus* died ten days after infection per os, eleven days after dropping culture into the conjunctival sac, and *leptospira* were cultivated from the organs of these animals. Icterus occurred in all species of *Meriones* and in *Gerbillus* and *leptospira* were cultivated from blood, liver and kidneys of all cases examined. *Microtus guentheri* behaved differently. In this vole the infection was not fatal and jaundice was never observed, although *leptospira* were found in the liver and kidneys.

Dog. A healthy dog, about a year old, was killed twenty-one days after subcutaneous injection of 2 ml of the third subculture isolated from the kidney of an experimentally infected calf. The agglutinin-titer rose above 1:4000 but at post-mortem examination no gross lesions were found and *leptospira* could not be cultivated from the liver and kidneys. The kidneys showed tubular degeneration and congestion, the liver had fatty changes, but in Levaditi-stained sections of these organs no *leptospira* were found.

Cat. A young cat infected simultaneously with the dog, excreted *leptospira* abundantly with the urine and when killed after twenty-six days showed an agglutinin titer of 1:2000: *leptospira* were cultivated in large numbers from its urine, but curiously not from the liver or kidneys. In histological sections glomerulonephritis was seen and several clumps of *leptospira* were present in the tubules.

Goats. An Arab goat in rather poor condition, infected subcutaneously with 4 ml. of the third subculture, aborted four days afterwards, became slightly icteric, produced dark-brownish urine on the sixth day, and died eight days after the injection. The body-temperature did not rise above normal. Post-mortem examination revealed the typical picture of leptospirosis and *L. grippotyphosa* was cultivated from the kidneys. A second, healthy Arab goat showed no symptoms of disease after infection with 3 ml of the fifth subculture isolated from the kidneys of the first goat. A high agglutinin-titer was produced (1:16,000). The blood-urea level remained normal. When slaughtered 46 days after infection, a large number of greyish pinpoint areas were seen on the surface of the kidneys; acute inter-

stitial nephritis and pericholangitis were found microscopically, but no leptospira were seen in the organs.

Sheep. Two Arab sheep infected at the same time as the goats remained clinically healthy but developed agglutinin-titers of 1:8.000 and 1:16.000 respectively. The second of these animals when slaughtered after 46 days, showed greyish spots in the kidneys similar to those found in the second goat, though in smaller numbers. Interstitial nephritis and pericholangitis were evident histologically but no leptospira were found. The cultures remained sterile.

Calf. A six months old calf infected with 4 ml. of culture subcutaneously developed agglutinins (titer 1:8000). Leptospira were cultivated from its blood four days later. By the twenty-fourth day the animal was so weak that it could hardly stand and was therefore slaughtered. No gross lesions and no jaundice were observed at post mortem examination. Very large numbers of leptospira were found in the urine. Leptospira were also seen in the liver and kidneys and they were cultivated from these organs and the urine. Microscopically the kidneys showed advanced tubular degeneration and acute interstitial nephritis, the liver acute and subacute pericholangitis. In Levaditi-stained sections of both organs a large number of leptospira were seen. The urea-content of the blood was 36 mg per cent. on 13.5.1952, 27 mg per cent. on 19.5., 15 mg per cent. on 26.5 and 60 mg per cent. on the day of slaughter (3.6). No bilirubin was found. The urine contained 800 mg per cent. urea.

DISCUSSION

The close antigenic relationship of the caprine strain to *L. grippotyphosa* strains which have caused serious outbreaks in man and dairy cattle in Israel, makes it probable that under favourable circumstances it also might become highly pathogenic for the latter and possibly also for sheep and other domestic animals. The results of experimental infection of a calf would support this opinion. Possibly, selected imported breeds might be more susceptible than native breeds.

The results of our investigations may explain the observations made in Switzerland, Silesia, Bavaria and other countries where leptospira agglutinins in significant concentrations have been detected in the blood of several cattle and other animals in localities in which the disease as such is unknown or very rare among them. In our case the goats acted as an indicator.

The course of the enzootic in different villages of the district, some of which were situated rather far apart from the others, can more satisfactorily be imputed to the presence of leptospira-excreting rodents than to contact with infected domestic animals. The high rate of infectivity and pathogenicity of the goat leptospira strain for several kinds of wild rodents is in favour of this supposition. Unfortunately, due to difficult local conditions, the epizootiological role of the field-rodents could not be investigated satisfactorily.

The epizootiological importance of dogs and cats in the spread of infection can not be excluded since several dogs in the area where the enzootic in goats occurred showed high agglutinin titres and an experimentally infected cat excreted the leptospira in its urine.

The spontaneous cessation of the outbreak in the spring of 1952 can partly be explained by gradually acquired immunity in the surviving animals. Atmospheric changes from the wet winter weather to the constantly dry sunny season may have contributed to the decline.

The great susceptibility for the goat strain shown by three species of native *Meriones* and by *Gerbillus* suggest that these rodents might be very useful for diagnostic and experimental purposes. Their susceptibility for other leptospira types is under investigation. *Meriones*, which in size lies between that of a hamster and a rat has important advantages as a laboratory animal. It is docile and does not bite, is easily managed and is fertile in captivity.

CONCLUSIONS

An outbreak of leptospirosis in Arab villages along the border of the Jordan State is described.

Considerable losses, amounting to 44 per cent. in one instance, occurred in goats although the infection sometimes remained inapparent.

The clinical symptoms were similar to those seen in cattle in Israel.

Increases in agglutinins to *Leptospira grippotyphosa*, the organism isolated from the goats, were detected in goats, sheep, cattle, and other species.

The experimental disease, set up in a number of animals, is described.

ACKNOWLEDGMENTS

The author is indebted to Dr. E. Cantor, District Veterinary Officer at Hedera, who was most helpful in supplying specimens for investigation and in providing epizootiological and clinical information; to Dr. T. Nobel of the Veterinary Institute at Tel Aviv, who carried out the histological examinations; to Mr. J. Naftali of the Plant-Protection-Service of the Ministry of Agriculture, for his valuable advice and assistance in acquiring field-rodents for experimental research; to Dr. F. Rappoport and Dr. A. Stark of the Hadassah-Hospital at Tel-Aviv for the chemical examination of blood and urine.

REFERENCES

- Freund, S., Trainin, D., and Malkin, M. (1941). *Refuah Veterinarith*, **8**, 153.
Hutyra, Marek and Manninger. (1949). *Special Pathology and Therapeutics of Diseases of Domestic Animals*. p. 182. Ballière, Tindall and Cox; London.

(Received for publication, September 18th, 1952).

STUDIES ON THE AVIAN LEUKOSIS COMPLEX*

2. SOME OBSERVATIONS ON THE BEHAVIOUR OF THREE
TRANSPLANTABLE AVIAN LYMPHOID TUMOURS

By

C. LE Q. DARCEL†

Animal Health Trust, Poultry Research Station, Houghton, Hunts.

INTRODUCTION

THE 'Avian Leukosis Complex' is a term under which a number of related diseases have been included (Jungherr, Durant and Lee, 1941). These are responsible for heavy mortality among poultry (Darcel, Lancaster and Gordon, 1952). Most of the losses arising from this group of diseases are due to the condition known as lymphomatosis which is held by most workers to be of a neoplastic-nature (Feldman and Olson, 1948; Jungherr, 1948; Engelbreth Holm, 1942). Lymphomatosis occurs in many forms, the visceral type of which closely corresponds to lymphoid leukaemia, lymphocytoma, lymphosarcoma and reticulo-sarcoma in human pathology.

Transmissible leukaemias, caused by specific viruses capable of producing these conditions, have been isolated from the leukaemic forms of avian leukosis known as erythroblastosis and myeloblastosis (Ellermann and Bang, 1908; Furth, 1929; Jarmai, 1930; Oberlin and Guérin, 1934). Similar pure strains of transplantable avian lymphoid tumours do not appear to have been isolated until 1940, although Furth (1933) isolated a strain which gave rise to lymphomatosis and occasionally myelomatosis and endothelioma. Pentimalli (1940, 1941) described a pure lymphoid tumour strain which was isolated from a spontaneous case of lymphomatosis. Similar strains have since been obtained by Olson (1941) and at the East Lansing Regional Poultry Laboratory in the United States by Burmester and Prickett (1945), Burmester and Denington (1947) and Burmester (1947a).

The three transplantable avian lymphoid tumours used in the present studies have been: (a) R.P.L. 12 isolated by Olson (1941); (b) R.P.L. 16 isolated by Burmester and Prickett (1945); (c) R.P.L. 19 isolated by Burmester and Denington (1947).

Olson (1941) has recorded the behaviour of tumour strain R.P.L. 12, perhaps better known as Olson's tumour, in the first 30 passages. Since then this tumour has been used for studies on tumour growth (Sharpless, Olson, Little and Davies, 1950), the presence of cell-free tumour agents (Burmester, 1947b; Burmester, Prickett and Belding, 1946a), and immunological studies (Burmester, 1947c; Burmester and Belding, 1947; Olson 1945a, b, c, 1946, 1947).

* These studies, which formed part of a thesis for the Ph.D. degree of the University of London, were supported by a grant from the A.R.C.

† Present address: Imperial Cancer Research Fund, Burton Hole Lane, Mill Hill, London, N.W.7.

Tumour strain R.P.L. 16 has been used by Burmester, et. al. (1946b), Emmel (1950), Carson (1951), and Davis (1952). Burmester (1947a) obtained strain R.P.L. 19 by the passage of the lymphomatous liver of a bird previously inoculated with a cell-free extract from a spontaneous case of lymphomatosis.

The cytological features of these three tumour strains have been well described by Olson (1941), Burmester and Prickett (1945), Burmester (1947a) and Emmel (1950). Briefly the cells are larger than lymphoid cells in normal lymphoid tissue, uniform in size and appearance with a vesicular nucleus with one or two nucleoli, and show a crescentic cytoplasm staining intensely blue with techniques employing methylene blue.

Following subcutaneous inoculations of chicks in the first few weeks of life a local tumour results with frequent metastasis to the viscera (Burmester, et. al., 1946a; Burmester and Prickett, 1945; Burmester, 1947a; Davis, 1952). The same high percentage of metastasis was not seen in the experiments of Emmel (1950), but the chicks used by him were older.

In the present paper we are presenting our own observations on the behaviour and growth of these tumour strains.

METHODS

With certain exceptions, the chicks used in the experiments were Barred Rock x Brown Leghorn crossbreds of both sexes, hatched on the premises. These were inoculated in the first week of life and usually at 1 to 2 days after hatching. The exceptions were the first few passages in which pure-bred and cross-bred chicks obtained from a local hatchery were inoculated at ages up to 21 days. In later experiments the effects of age, sex, breed and induced resistance to tumour growth were studied. The chicks were accommodated in small cloche-type brooders, heated by electric light bulbs, holding 10 to 15 chicks up to 4 weeks of age. The food fed was a chick mash complying with national standards.

Throughout these experiments tumour minces of the required concentration in physiological saline were prepared with a special mincer, (Craigie, 1949a) from livers of chicks dying as a result of inoculation with these tumours. The suspensions were sufficiently fine for inoculation from an insulin syringe through a 20 gauge hypodermic needle. Except where otherwise stated, a standard dose of 0.25 ml. of a suspension prepared by mincing 1 part by weight of tumour with nine parts of physiological saline, was inoculated subcutaneously into the area immediately overlying the pectoral muscle.

After the early transmission experiments chicks were regularly palpated for the first appearance of tumours. The rapidity of growth is such that although the tumour is often small when first detectable, by the following day it will have considerably increased in size confirming the previous day's palpation. If a 'tumour' cannot be

palpated the following day, its record as a positive palpation is cancelled.

Mean 'latent periods' are calculated on the basis of those chicks in which tumours appear, whilst mean survival times are estimated on the chicks dying during the course of the observation period, which has normally been 28 days. Subsequently, the latent period will be referred to as the TFA and the time that chicks die after the inoculation as the ST.

Aseptic autopsies have been performed on all chicks dying and tumour involvement at the site of inoculation with or without metastasis to the liver noted. When the latter organ is involved, it is easily recognised by its marked enlargement, colouring and friable nature. Post-mortem decomposition rapidly takes place in chicks not collected from the cloches immediately after death, and material from birds in which there has been evidence of such changes has not been used for passage. No systematic examination of organs other than the liver for tumour involvement has been undertaken.

Chicks dying but showing no evidence of tumours at autopsy are deleted from the records. Such deaths have been rare (1 to 2 per cent.) and have occurred from causes such as ruptured or infected yolk sacs.

EXPERIMENTAL OBSERVATIONS

The establishment of tumour strains R.P.L. 12, 16 and 19

Burmester, Brandly and Prickett (1944), Burmester (1950), have shown that avian lymphoid tumours when stored en masse remain active at -40°C to -70°C . Sealed pyrex tubes of tumour material that had been stored in this manner for considerable periods (see Table I) were embedded in a 30 lb. block of CO_2 ice and despatched by air to this laboratory from the East Lansing Regional Laboratory. On arrival the contents were thawed and were macerated with a pestle and mortar with 4 parts of saline, with the exception of the material which was claimed to be a cell-free extract of R.P.L. 12. The results of the inoculation into young chicks in 0.2 to 0.5 ml. amounts are shown in Table I. It will be seen that the cellular suspensions prepared with R.P.L. 12, killed all chicks inoculated in 9.63 ± 0.94 days; with R.P.L. 16, six out of ten chicks died after 14.0 ± 3.48 days; and with R.P.L. 19, three out of six chicks died after 22.0 ± 0 days. All chicks developed pectoral tumours following the inoculations, except the three dying after inoculation with R.P.L. 19. All showed tumour involvement of the liver, but one chick inoculated with R.P.L. 12 showed in addition lesions of the heart and proventriculus.

Tumour material from strains R.P.L. 16 (tube 3313), and 18 (tube 3366) serially passaged with cell-free material, failed to produce tumours within the period of observation. R.P.L. 18 was another lymphoid tumour strain isolated by Burmester (1947a). The cell-free extract of R.P.L. 12, prepared by Dr. Burmester killed

TABLE I

THE ACTIVITY OF TUMOUR MATERIAL OBTAINED FROM EAST LANSING REGIONAL POULTRY LABORATORY, WHERE IT HAD BEEN STORED AT LOW TEMPERATURE.

<i>Tube No.</i>	<i>Tumour Strain</i>	<i>No. of times previously passaged</i>	<i>Days stored</i>	<i>Material</i>	<i>Route Inoculated</i>	<i>Age of chicks inoculated (days)</i>	<i>No. of chicks inoculated</i>	<i>No. of chicks developing tumours</i>	<i>Mean survival time</i>
2142	RPL 12	144 C	801	Pectoral tumour	Sub-cutaneous	14	8	8	9.63 ± 0.94
2200	RPL 16	61 C	798	Pectoral tumour	Sub-cutaneous	14	10	6	14.00 ± 3.48
1009	RPL 19	3 C	1454	Tumourous liver	Sub-cutaneous	7	6	3	22.0 ± 1.00
3239	RPL 12	10 F	164	Cell-free extract of tumourous livers	Intra-peritoneal	7	4	4	29.0 ± 0
3313	RPL 16	4 F	102	Tumour liver	Intra-peritoneal	7	15	0	—
3366	RPL 18	10 F	83	Tumourous liver	Intra-peritoneal	7	4	0	—

C. Tumour strain previously passaged using tumour cell-containing inocula.

F. Tumour strain previously passaged using cell-free inocula.

TABLE 2

THE BEHAVIOUR OF CHICKS INOCULATED WITH TUMOUR STRAINS RPL 12, 16 AND 19 IN THE FIRST TWENTY-FIVE TRANSMISSION EXPERIMENTS

<i>Tumour Strain</i>	<i>Chicks inoculated</i>	<i>Chicks dying</i>	<i>% Mortality</i>	<i>Mean ST in days</i>	<i>Coefficient of variation</i>	<i>Median</i>	<i>Range</i>
RPL 12	130	124	95.40	9.54 ± 0.25	29.30	7.27	6-20
RPL 16	128	123	95.35	8.47 ± 0.24	31.31	7.54	5-22
RPL 19	129	119	92.25	10.10 ± 0.40	43.45	8.45	4-24
All	388	366	94.35	9.36 ± 0.19	38.45	7.67	4-24

four chicks inoculated intraperitoneally in 29 days, with enlargement of the liver. This latter material had been prepared from tumourous livers macerated in a Waring Blendor and then centrifuged at 3,000 R.C.F. and then 30,000 R.C.F., the last supernatant being retained. Further passage into young chicks of suspensions of the enlarged livers prepared from the chicks dying following the inoculation of this extract, did not result in further tumours.

Serial passages

It was felt desirable to compare the behaviour of these tumours in the crossbreeds available here with the results of observations of the earlier workers already mentioned. The results of the first twenty-five transmission experiments with each strain have been summarized in Table 2. A total of 388 chicks were inoculated of which 94.53 per cent. died. Deaths followed the almost invariable involvement of the liver which was recognised macroscopically as being affected in 332 or 90 per cent. of the 366 chicks dying with

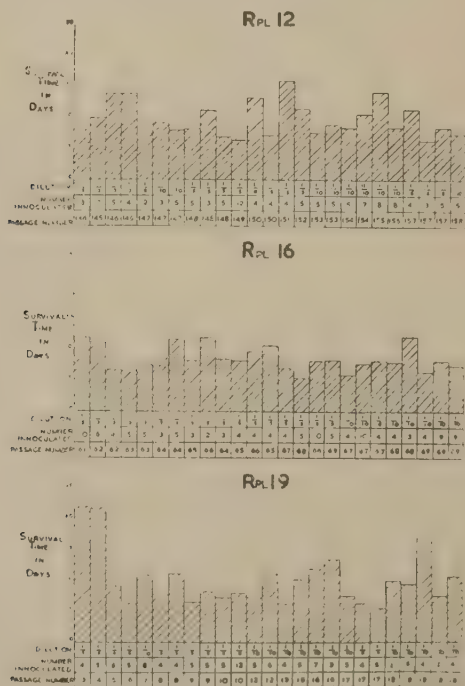


DIAGRAM I

Variation in the mean survival time in the first twenty-five experiments with tumour strain RPL 12, 16 and 19.

tumours. The affected liver normally showed diffuse enlargement and in only two chicks were nodular liver tumours recognised. The heart, lungs, proventriculus, spleen and adrenals were less frequently recognised as being macroscopically affected.

The range over which deaths occurred was considerable, being 6 to 20 days for R.P.L. 12, 5 to 22 days for R.P.L. 16, and 4 to 24 days for R.P.L. 19. The variations that occurred in the mean survival times from passage to passage were partly responsible for the wide range (Diagram 1). This variation in mean survival time also occurred in experiments involving the inoculation of suspensions from the livers of different chicks of the same tumour passage. For instance in experiments T153, 154, and 159 in which 4, 3 and 5 chicks respectively were inoculated with the 295th passage of R.P.L. 12 (157th passage as R.P.L. 12 plus 138 passages by Olson prior to its acquisition by East Lansing), the mean survival times were 11.50 ± 0.95 , 6.67 ± 0.33 and 8.60 ± 1.33 days. In the case of experiments T153 and 154 the difference is probably significant, notwithstanding the small numbers of chicks employed.

The palpation of tumours

Since daily palpation has been practised for the first appearance of tumours in the experiments to be described later, some knowledge of the error involved was essential. The possible subjective error was investigated in experiment T394, and the results are shown in Table 3.

TABLE 3

EXPERIMENT T394 SHOWING SUBJECTIVE DIFFERENCES OF THREE INDIVIDUALS IN DETERMINING THE LATENT PERIOD FOR TUMOURS TO APPEAR

<i>Individual</i>	<i>Palpation Series</i>	<i>Total number of tumours palpated</i>	<i>Mean TFA</i>
A	1	32	6.56 ± 0.156
	2	33	6.55 ± 0.155
B	3	25	6.76 ± 0.183
	4	27	6.85 ± 0.146
C	5	34	6.37 ± 0.143
	6	34	6.47 ± 0.142

In this experiment the chicks were palpated in turn for 8 days by each of three individuals who examined the chicks twice and recorded the results separately. To minimise subjective error no communication between individuals was permitted. Chicks with tumours were

picked out, identified and recorded as positive, only after the remainder of the chicks had been palpated. Thirty four chicks were inoculated with tumour suspension and 34 uninoculated chicks randomised with them. No false positives were recorded amongst uninoculated chicks, by any of the three individuals. Close agreement between the results of the two series of palpations by each individual was observed. Larger differences were observed between the mean latent period as determined by each of the three individuals. Statistically, however, these differences were of a very low order of significance.

The development of palpable tumours in relation to the disease process

Three experiments were made in which groups of chicks were inoculated with the same dose of tumour suspension prepared from each of the three tumour strains. Chicks were palpated daily and small numbers (3 to 4) in each group were killed at regular intervals. These were autopsied and pieces of liver fixed in Bouin's fixative (Allen's, PFA₃ modification) and embedded in paraffin. The livers were sectioned and stained with Ehrlich's haematoxylin and eosin for evidence of metastasis to the liver.

In experiment T353 all tumours that were palpated prior to the chick being killed developed on the third day following inoculation. Microscopic involvement of the liver with the appearance of isolated tumour cells in the sinusoids was observed for the first time on the 5th day, 2 days after the development of the pectoral tumours. Involvement of the liver detectable by macroscopic examination at autopsy with a histological picture of the type shown in Fig. 1 (shown in comparison with a normal liver Fig. 2) occurred on the 7th day, on which day deaths occurred for the first time. Results with R.P.L. 16 and 19 were similar (Darcel, 1952).

With regard to the mechanism by which metastasis takes place, there are two possible alternatives, (a) that dislodgement of tumour cells into the bloodstream occurs from involved blood-vessels in the pectoral tumours (see Fig. 3), which finally lodge in the liver sinusoids, and (b) that virus liberated from the primary tumour, induces tumourous change in the primitive reticulo-endothelial cells in the liver sinusoids. If the first occurred it would be expected that shortly after the appearance of a pectoral tumour, tumour cells would be found in significant numbers in the bloodstream. An experiment to test this was made with tumour strain R.P.L. 16 in which total white cell counts and differential counts were made on daily blood samples from the wing vein of five inoculated and five uninoculated chicks. Although palpable tumours developed in the inoculated chicks on the sixth day, and five died, one on the eighth day and four on the ninth day with tumour infiltration of the liver, no evidence of a true leukaemia was obtained. A rise in lymphocytes occurred but this did not reach a high level and was accompanied by a rise in heterophils. No increase in the number of

haemocytoblasts or other primitive cells occurred in the blood of these birds, and the frequency was too low to be shown in Diagram 2 which illustrates the levels of lymphocytes and heterophils observed.

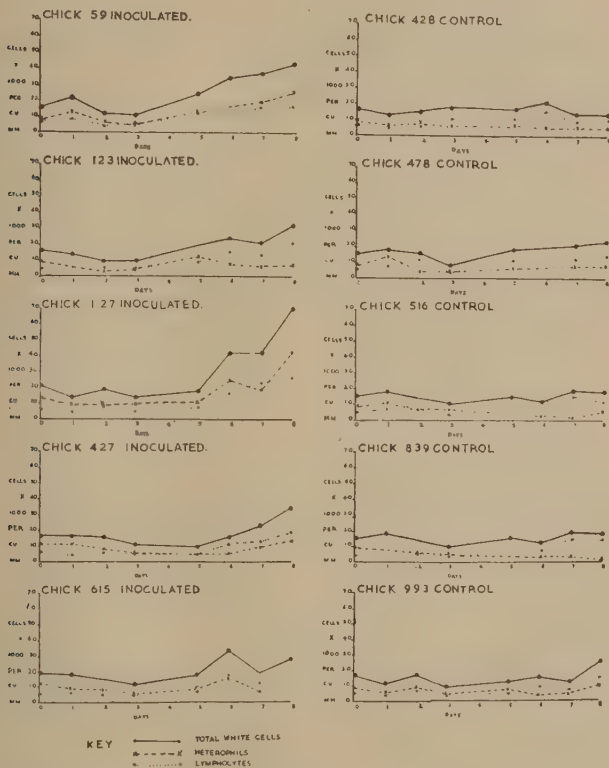


DIAGRAM 2

Total daily white cell, heterophil and lymphocyte counts in five control and five tumour inoculated chicks.

In other experiments attempts were made to demonstrate the transition of reticulo-endothelial cells in the liver to tumour cells by the use of vital dyes. Owing to technical difficulties this attempt to confirm (b) proved unsuccessful (Darcel, 1952).

The relationship between TFA and ST

The results of the inoculation of large groups of chicks with different suspensions of the same tumour strains is illustrated in

Table 4. The TFAs have been plotted against the STs and the calculated regression line drawn in Diagram 3. The close linear

TABLE 4
RESULTS OF EXPERIMENTS T282-T367

<i>Expt. No.</i>	<i>Tumour Strain</i>	<i>No. of chicks inocu- lated (T)</i>	<i>Measure- ment η</i>	<i>No. of observa- tions</i>	η $\frac{\eta \times 100}{T}$	<i>Mean in days</i>	<i>Range in days</i>	<i>Observa- tion period in days</i>
T282	RPL 19	40	TFA	39	97.5	10.77 ± 0.02	9-13	44
			ST	33	82.5	18.00 ± 0.89	12-32	
T290	RPL 19	40	TFA	39	97.5	8.49 ± 0.14	6-10	30
			ST	35	87.5	12.51 ± 0.59	10-25	
T301	RPL 12	40	TFA	34	85.0	6.27 ± 0.09	6-8	9
			ST	40	100.0	7.87 ± 0.10	7-9	
T302	RPL 12	38	TFA	34	89.5	7.65 ± 0.13	7-8	20
			ST	37	97.4	10.87 ± 0.35	10-20	
T311	RPL 12	40	TFA	40	100.0	5.21 ± 0.08	4-6	9
			ST	40	100.0	7.98 ± 0.25	7-9	
T313	RPL 16	41	TFA	32	72.5	13.13 ± 0.45	8-20	31
			ST	14	34.1	19.29 ± 1.41	13-29	
T314	RPL 19	40	TFA	19	47.5	14.12 ± 0.27	10-20	31
			ST	8	20.0	20.25 ± 1.10	15-25	
T315	RPL 16	37	TFA	37	100.0	6.62 ± 0.11	5-25	24
			ST	37	100.0	11.35 ± 0.56	9-24	
T316	RPL 12	38	TFA	38	100.0	6.00 ± 0.14	5-8	10
			ST	38	100.0	9.40 ± 0.08	8-10	
T319	RPL 19	40	TFA	35	87.5	4.25 ± 0.08	4-5	8
			ST	40	100.0	6.80 ± 0.10	5-8	
T321	RPL 16	36	TFA	36	100.0	6.42 ± 0.15	5-8	31
			ST	34	93.9	14.88 ± 0.94	9-27	
T362	RPL 16	40	TFA	37	92.5	4.32 ± 0.09	3-5	10
			ST	40	100.0	7.53 ± 0.05	6-10	
T367	RPL 16	40	TFA	40	100.0	3.67 ± 0.12	3-4	33
			ST	39	97.5	7.72 ± 0.10	7-9	

TFA: Time of first appearance of tumours in days after inoculation.

ST: Time of survival of chick in days after inoculation.

correlation of the two observations is well shown. The coefficient of correlation between the two observations was found to be 0.93 with a high degree of significance > 0.001 . Considerable deviation from the regression occurred in one experiment (T321).

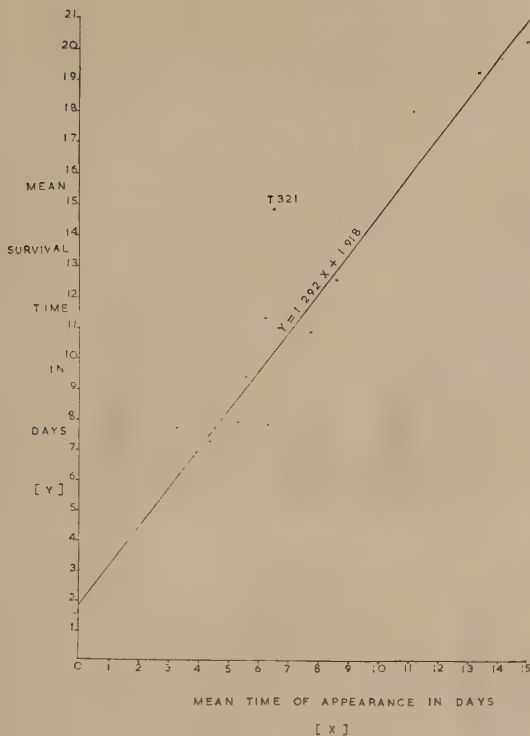


DIAGRAM 3

Showing the close agreement between the experimental observations and the calculated regression of mean survival time and mean time for first appearance of tumours. Each observation is based on the mean response of 35-40 chicks inoculated with the same tumour dose.

The variation of the individual response

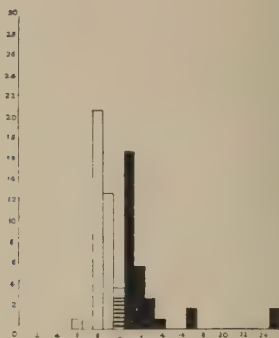
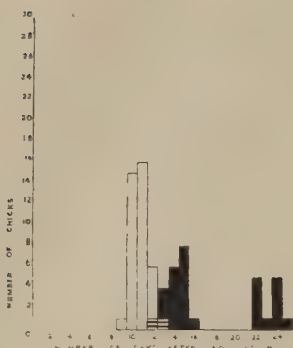
In Diagram 4 the TFAs and STs are shown for individual chicks following the same dose of tumour suspension (strain R.P.L. 19). It will be seen that in experiment T319 tumours appeared and deaths occurred over a small range. In experiment T282, T290 and T314 the range over which these observations occurred was greater. Identical behaviour was seen in similar experiments with R.P.L. 16.

In the four experiments with R.P.L. 12, details of which are given in Table 4, the ranges over which deaths occurred and tumours appeared was similar to that in experiment T319 with R.P.L. 19. These wide ranges observed in certain experiments suggest wide differences in the rate of tumour growth in different individuals.

RPL 19

T. 282

T. 290



T. 319

T. 314

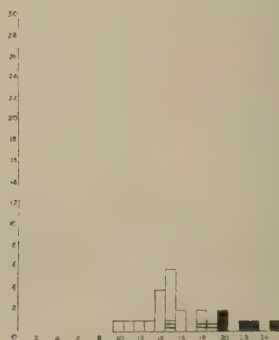


DIAGRAM 4

Variation in individual response to the same tumour dose following the inoculation of Rpl. 19, into groups of 35-40 chicks. Unshaded columns show the number of chicks developing tumours for the first time each day after inoculation. Black columns show the number of chicks dying each day after inoculation. The broken-shaded areas represent coincidence of observation: for instance in T.282 on the 12th day, 6 tumours were detected for the first time and 1 chick died whilst, on the 13th day, only one more tumour was palpated and 4 chicks died.

Individual variability in response to tumour inoculation is seen in experiments in which the same dose of tumour suspension produces the death of some chicks following growth of tumour whilst in others the tumour grows for a while and then disappears.

This has been well demonstrated in the experiments in which the inoculation was postponed until the birds were 56 days old. The details of these experiments are given later.

The effect of sex on the response of young chicks to the implantation of the same tumour dose

Since chicks of both sexes had been used in the foregoing experiments, it was of interest to determine whether sex had any effect on the response of young chicks to tumour inoculation. In experiments T362 and T367 shown in Table 4, the chicks were sexed on a basis of the feathering (sex-linked in the Barred Rock x Brown Leghorns) and the differences in tumour response noted. In experiment T362 the males developed tumours in 4.16 ± 0.12 days and the females in 4.50 ± 0.12 days, and died after 7.70 ± 0.15 days and 7.35 ± 0.18 days respectively. In experiment T367 the males developed tumours in 3.70 ± 0.11 days and the females in 3.65 ± 0.16 days, and died after 7.84 ± 0.15 days and 7.60 ± 0.18 days respectively. Thus the behaviour of the two sexes in these two experiments showed no significant difference.

The effect of environment

In the experiments summarised in Table 4, the groups of 40 birds were divided into smaller groups of ten, which were reared in separate cloche brooders. This afforded an opportunity for determining whether small unrecognised environmental differences would have any significant effect on tumour growth. This was felt to be of particular importance in the design of experiments in which subdivision into small groups is necessary as in the studies on the effect of age on tumour response. Circumstances such as these are also met with in experiments in which a drug is administered in the food or water. Experiment T315 was typical of the present series. The mean TFAs for the four groups were 6.50 ± 0.27 , 6.90 ± 0.18 , 6.38 ± 0.18 and 6.67 ± 0.24 days; whilst the mean STs were 11.80 ± 1.37 , 12.50 ± 1.21 , 10.63 ± 0.42 and 10.33 ± 0.29 days respectively. Whilst the differences in mean TFAs were small, the STs showed larger differences which were probably significant. Differences of a low order of significance, particularly with STs, were large enough to justify randomisation where possible.

The effect of breed on the growth of tumours

It was important to determine whether tumour growth proceeded at different rates in pure bred chicks of the two strains from which the cross-breds used were derived. Had a difference existed, it would have stressed the need for the routine use of pure-bred chicks in quantitative studies, as crossing might then have contributed to the individual variation in response already noted. The results of experiments in which the possibility of breed affecting the tumour

responses are shown in Table 5. No significant differences were observed in the behaviour of the Barred Rocks, Brown Leghorns or the cross resulting from the mating of Brown Leghorn cocks to Barred Rock pullets.

TABLE 5

TIMES OF APPEARANCE OF TUMOURS AND SURVIVAL TIME IN DAYS IN PURE AND CROSS-BRED STRAINS OF CHICKS

Expt. No.	Tumour	Chicks used	Chicks Inoculated	Tumours palpated		Deaths	
				Tumours palpated	Latent period	Deaths	Survival time
T.359	RPL 12	Crossbreds	10	10	3.80 $\pm$ 0.13	10	6.60 $\pm$ 0.16
		Brn. Leghorns	10	10	3.80 $\pm$ 0.13	10	6.20 $\pm$ 0.13
T.360	RPL 16	Crossbreds	10	10	7.60 $\pm$ 0.26	10	11.90 $\pm$ 0.62
		Brn. Leghorns	10	10	7.00 $\pm$ 0	6	13.33 $\pm$ 1.15
T.379		Crossbreds	10	10	5.00 $\pm$ 0.26	10	8.70 $\pm$ 0.15
		Brn. Leghorns	10	10	4.60 $\pm$ 0.16	10	8.50 $\pm$ 0.17
T.384		Crossbreds	10	10	3.60 $\pm$ 0.16	10	8.10 $\pm$ 0.35
		Brn. Leghorns	10	10	4.30 $\pm$ 0.30	10	8.00 $\pm$ 0.30
T.504	RPL 12	Crossbreds	9	8	9.13 $\pm$ 0.30	8	14.50 $\pm$ 1.21
		Barred Rocks	10	10	9.60 $\pm$ 0.34	10	13.22 $\pm$ 0.33

The effect of route of inoculation on tumour response

No systematic study has been made on the influence of route of inoculation on the tumour response. In experiment T415 suspensions of tumour strain R.P.L. 16, at concentrations in saline of 10^3 and 10^4 were inoculated by intraperitoneal or subcutaneous routes into groups of 9 to 10 chicks. Following the inoculation of the 10^3 suspension, tumours developed in 9.78 ± 0.14 and 8.90 ± 0.28 days and all the chicks died after 13.33 ± 0.85 and 14.25 ± 0.72 days respectively in the chicks inoculated by the intraperitoneal or subcutaneous routes. Following the inoculation of the 10^4 suspension tumours developed in 11.67 ± 0.43 and 11.00 ± 0.37 days whilst 10 out of the 19 chicks inoculated died after 14.20 ± 1.02 and 16.00 ± 0.95 days after the intraperitoneal and subcutaneous inoculations respectively. The general effect was thus for tumours to be palpated earlier but for chicks to die later when inoculated by the subcutaneous route.

The effect of age on tumour response

Age is a factor known to influence the tumour response of other transplantable tumours and a series of experiments was made in which the growth of these lymphoid tumours in older birds was compared with that in young chicks. The results are illustrated in

Table 6. Cross-bred chicks were used in all experiments, with the exception of T₅₁₉ in which the five adult cocks used included 1 Brown Leghorn, 1 Ancona, and 3 Light Sussex.

TABLE 6

EFFECT OF AGE OF CHICKS ON THE RESPONSE TO THE TUMOURS INOCULATED

Expt. No.	Tumour	Birds Inoculated	Age in days	Tumours palpated		Deaths	
				Tumours palpated	Latent period in days	Deaths	Survival time in days
T.428	RPL 19	10	2	9	9.78 ± 0.40	10	14.40 ± 0.95
		9	38	8	8.75 ± 0.16	8	19.75 ± 1.73
T.359	RPL 12	10	2	10	3.80 ± 0.13	10	6.60 ± 0.16
		10	8	10	4.00 ± 0	9	8.56 ± 0.56
		8	22	8	4.25 ± 0.60	6	11.83 ± 1.49
T.346	RPL 19	9	6	7	7.00 ± 0.44	7	10.71 ± 0.57
		10	13	9	5.33 ± 0.24	7	13.38 ± 0.56
T.453	RPL 12	7	2	5	3.60 ± 0.24	7	5.80 ± 0.20
		8	56	8	6.25 ± 0.41	3	19.00 ± 1.15
T.454	RPL 19	7	2	7	6.86 ± 0.14	7	8.00 ± 0.96
		9	56	9	8.00 ± 0.96	2	18.00 ± 1.00
T.455	RPL 16	7	2	7	4.43 ± 0.30	7	7.29 ± 0.42
		9	56	9	6.33 ± 0.37	4	26.25 ± 4.46
T.519	RPL 19	5	9	5	6.60 ± 0.24	5	10.00 ± 0
		5	Adult cocks	4	8.75 ± 1.18	2	14.50 ± 3.50

Examination of the data shows that in the three experiments made up to T₃₄₆, the differences in the mean time of the first appearance of tumours and mean survival time were small. In view of the size of the standard error these differences are of a low order of significance. In birds inoculated when 56 days of age, striking differences in the behaviour of the tumour implants compared with that in young chicks were observed with all three tumour strains. Tumours appeared later and fewer birds died and the survival time of these was considerably longer in the older birds. All the older birds developed palpable tumours and with the exception of the birds dying as the result of tumour implantation, the growths had completely regressed within a month. In those birds dying as the result of tumour inoculation, pectoral tumours reached a considerable size. Although inoculated subcutaneously, growth proceeded primarily within the muscle tissue. Plate 6 shows a cross section of such a pectoral tumour from a bird that died in experiment T₄₅₃ following inoculation with R.P.L. 12. Diffuse involvement of the proventriculus was seen in 7 out of the 9 birds inoculated at 56 days dying in experiments T₄₅₃ to T₄₅₄. In the 2 day old chicks which

died following the inoculation with the same tumour suspension, no such involvement of the proventriculus was seen. One of the birds dying after inoculation with R.P.L. 16 showed enormous enlargement of the pancreas.

Tumours appeared in four of the five cockerels inoculated at a year old with strain R.P.L. 19, but only two died after 14.50 ± 3.50 days. Five cross-bred chicks inoculated at 9 days old with material from the same suspension died after an average period of 10.00 ± 0 days. In the two adult birds dying, pectoral tumours did not reach any considerable size and growth within the pectoral muscle was not comparable to that observed in the birds dying following inoculation at 56 days of age. Sections of the pectoral muscle of one bird showed infiltration of tumour cells between the muscle fibres (Fig. 4) whereas section of pectoral tumour in the other showed nodular infiltration (Fig. 5). One of the birds which died exhibited gross enlargement of the liver, while the other succumbed from an involvement of the mesentery with numerous small button-like tumours. The liver in the latter bird was not diffusely involved, but the surface was completely replaced with red button-like tumours: sections showed focal tumours completely localised to the surface consisting of cells typical for these lymphoid tumour strains.

Induced Resistance

Five of the eight birds in T₄₅₃ inoculated with R.P.L. 12 developed palpable tumours which completely regressed within 31 days. In T₅₀₃, not shown in the tables, these birds first inoculated at 56 days of age were again inoculated 42 days later with a standard dose (0.25 ml. of a 1/10 suspension of tumour involved liver) of R.P.L. 19. Nine control birds were inoculated at 6 days of age: 8 developed tumours at an average of 6.25 ± 0.16 days after inoculation and 9 died, 9.67 ± 0.33 days after inoculation. None of the birds which had been previously inoculated with R.P.L. 12, developed tumours or died as the result of inoculation. Similarly, the seven survivors of T₄₅₄ previously inoculated with strain R.P.L. 19 and the five survivors of T₄₅₅ previously inoculated with strain R.P.L. 16 did not develop tumours or die following re-inoculation with the R.P.L. 19 suspension used in T₅₀₃, 42 days after the previous inoculation. Whilst these results suggest that previous recovery from inoculation with any of the three tumours produced a complete immunity to strain R.P.L. 19, the evidence in the previous section suggested there is an enhanced resistance to tumour growth associated with age. In order to control this factor the experiment was repeated in the following manner.

The birds which had survived the original inoculations with the three tumour strains in experiments T₄₅₃, T₄₅₄ and T₄₅₅ and which failed to develop tumours in experiment T₅₀₃ when re-inoculated with R.P.L. 19, again failed to develop tumours when re-inoculated at 114 days of age with this tumour. The last suspen-

sion produced tumours in young chicks and old cockerels (T519, Table 6), used as controls. These results suggest that in the previous experiments failure to develop tumours after re-inoculation with R.P.L. 19 was not solely due to the increase in age but was attributable to recovery from the inoculation of tumours R.P.L. 12, 16 and 19 respectively.

DISCUSSION

The behaviour of these tumours in the passage experiments described was in close agreement with the findings of earlier workers (Olson, 1941; Burmester and Prickett, 1945; Burmester, 1947a; Davis, 1952). The lower percentage of metastases occurring in Emmel's experiments (Emmel, 1950) has been demonstrated in our own experiments as probably a manifestation of age resistance.

Small but significant differences in the behaviour of R.P.L. 12, 16 and 19 were observed in our experiments. They were less well-marked, however, than those observed by the above mentioned workers and this was perhaps due to the increased number of passages which the strains had by then undergone.

Significant differences in survival times were noted in our early transmission experiments. Close correlation was found in other experiments between the TFA and ST. These differences in the TFA and ST from experiment to experiment suggest that these two quantities may be used as expressions of the tumour producing activity of suspensions. That this is possible is suggested by the findings of Gottschalk (1943, 1946), Craigie (1949b) and Eckert, Beard and Beard (1951) who established linear relationships between the latent period or its reciprocal and the logarithm of the dose inoculated. This observation has formed the basis of experiments in which this relationship was confirmed for the tumours described in this article (Darcel, 1952).

The appearance of isolated tumour cells in the liver shortly after tumours are detected by palpation suggests that these are carried by the blood stream. No evidence of a true leukaemia was found, however. In contrast, in a small number of cases, smears of heart blood taken from birds after death have shown an appearance suggestive of leukaemia. This may have been an artefact following post-mortem clotting and differential cell sedimentation. Burmester and Prickett (1945) also failed to demonstrate a significant rise in lymphocytes until shortly before death.

In the absence of a leukaemia it is difficult to account for the rapid metastasis of these tumours, although tumour cell involvement of blood vessel walls in both the liver and pectoral tumours was frequently observed. If, however, viruses exist in these tumours, as claimed by Burmester (1947a, b) there is another mechanism by which the viscera might be involved. More detailed studies of the tumour producing ability of the blood at different stages in the disease are required.

The histological picture shows that the distribution of tumour cells in the liver is almost completely intravascular*, the cells lying within the hepatic sinusoids. In the spontaneous disease the lesions are, in contrast, almost always extravascular, (Olson, 1941).

Palpation for the first appearance of tumours has been criticised by some workers as subject to too large a subjective error to be used as a measurement in experimental studies (Shear, Imagawa, Syverton, and Bittner, 1951). Nevertheless, in the present experiments with these lymphoid tumours, the subjective error was so small as to justify palpation as a simple criterion of tumour growth.

There is no evidence in our experiments that the use of pure-bred strains reduced the variation in individual response observed in the cross-breds. However, these experiments were made with young chicks, and the failure to demonstrate differences in tumour growth in different breeds and the two sexes may arise out of the youth of the chicks inoculated. Gross (1950) showed that suckling mice of an inbred strain completely resistant as adults to a strain of mouse leukaemia, were completely susceptible. The slower growth of the fowl tumours in older birds may be the result of the known ability of older birds to produce antibodies more readily than chicks (Wolfe and Dilkes, 1947). The failure of birds that had previously recovered from tumour implants to develop tumours on re-inoculation confirms the experiences of Burmester and Belding (1947). Our own experiments demonstrated cross immunity between the three strains, thus confirming the findings of Burmester and Belding. By reversing the order of inoculation these workers were, however, able to demonstrate antigenic differences between the strains studied by them.

The present studies have formed a basis for experiments in which the effect of varying the tumour dose was investigated. The rapidity of growth, and the close correlation between the latent period and survival times suggests the suitability of these tumour strains for quantitative studies. Further, in view of their origin these strains should prove suitable for the routine screening of drugs for tumour inhibitory properties, with a view to the possible control of lymphomatosis in the field, by chemotherapy.

SUMMARY

The behaviour of three transplantable avian lymphoid tumours (R.P.L. 12, 16 and 19) has been studied in experiments utilising cross-bred chicks inoculated for the most part in the first few weeks of life.

In the first twenty-five passages these strains killed chicks with a frequency of 95.4 per cent., 95.55 per cent. and 92.35 per cent. after 9.54 ± 0.25 , 8.47 ± 0.24 and 10.10 ± 0.40 days, respectively.

* Recent observations by the writer suggest that this intravascular appearance may be an artefact and that tumour cells lie outside the hepatic sinusoids.

A linear correlation was demonstrated between this mean time for tumours to develop (in the determination of which the subjective error of palpation was found to be small) and the mean time taken for the tumours to kill inoculated chicks.

At post-mortem the macroscopic lesions found, which were characterised by the development of a tumour in the subcutaneous muscular tissues at the site of inoculation and visceral lesions of the liver, proventriculus and other organs were indistinguishable in the three strains: moreover, the microscopic appearance of the pectoral tumours and the affected livers showed no differences. In all cases growth in the liver was largely intravascular and confined to the sinusoids. Involvement of the walls of large blood vessels, was, however, often observed.

Tumour cells appeared in the liver sinusoids 1 to 2 days after the appearance of the pectoral tumours. Since tumour cells were not detectable in the blood during this period and no leukaemia was observed, the mechanism by which tumour cells develop in, or reach the liver is still unknown.

Breed and sex failed to influence tumour growth in young chicks.

Palpable tumours developed in the abdominal wall following intraperitoneal inoculation. Following inoculation by this route, tumours were palpable later and chicks died sooner, than after subcutaneous inoculation.

In birds over 56 days of age when inoculated, the tumours appeared later, the death rate was lower and the survival time longer, than in young chicks inoculated with the same tumours. The proventriculus was more frequently involved than in younger chicks.

Birds recovering from a previous inoculation with the three tumour strains failed to develop further tumours when inoculated on two further occasions with suspensions of R.P.L. 19, which were highly active in young chicks.

Small unrecognised differences in environment may influence tumour growth and suggest the necessity for careful randomisation of chicks or repetition of experiments where the effect of the administration of drugs or other substances on tumour growth is to be determined.

ACKNOWLEDGMENTS

The author takes this opportunity of thanking Mr. M. Wilson and Miss A. Jackson for their technical assistance, Dr. Nils Lannek for his co-operation in the haematological studies, Dr. B. R. Burmester of East Lansing, Michigan, U.S.A., who provided the three strains and Mr. J. Patman of the School of Pathology, Cambridge for the microphotography.

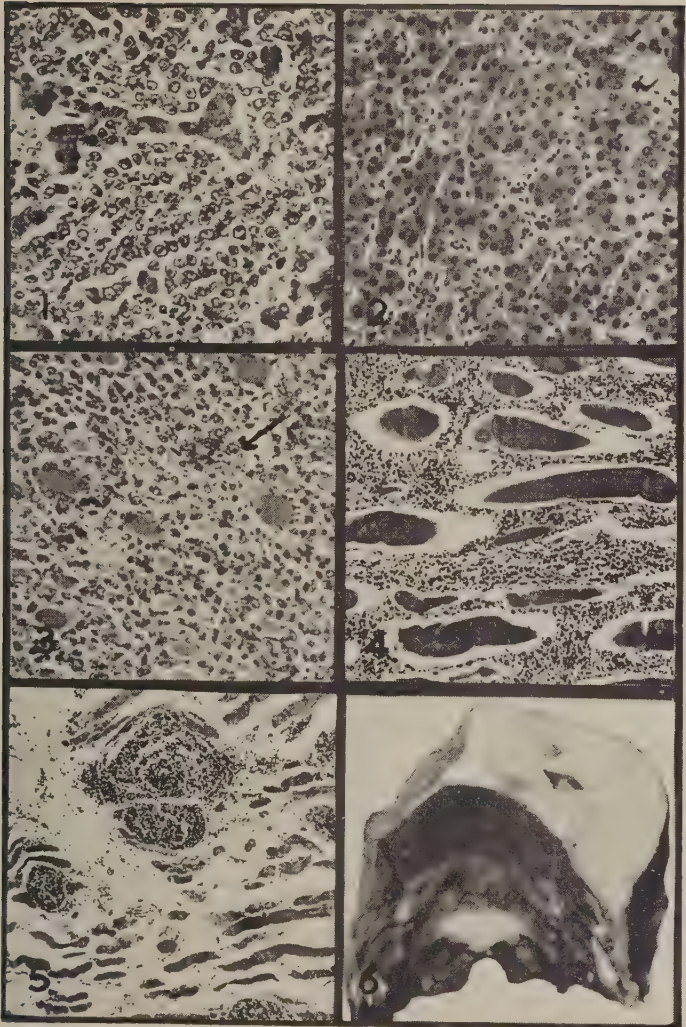
REFERENCES

- Burmester, B. R. (1947a) *Cancer Res.*, **7**, 786; (1947b) *Poult. Sci.*, **26**, 215; (1947c) *Cancer Res.*, **7**, 459; (1950) *Ibid.*, **10**, 708.
Burmester, B. R. and Belding, T. C. (1947) *Amer. J. vet. Res.*, **8**, 128.
Burmester, B. R. Brandly, C. A. and Prickett, C. O. (1944) *Proc. Soc. exp. Biol. N.Y.*, **55**, 203.

- Burmester, B. R. and Denington, F. M. (1947) *Cancer Res.*, **7**, 779.
- Burmester, B. R. and Prickett, C. O. (1945) *Poult. Sci.*, **5**, 652.
- Burmester, B. R. Prickett, C. O. and Belding, T. C. (1946a) *Cancer Res.*, **6**, 189; (1946b) *Poult. Sci.*, **25**, 616.
- Carson, J. B. (1951) *Ibid.*, **30**, 213.
- Craigie, J. (1949a) *Brit. J. Cancer*, **3**, 249; (1949b) *Brit. med. J.*, *ii*, 1485.
- Darcel, C. le Q. (1952). *Thesis*. University of London.
- Darcel, C. le Q., Lancaster, J. E. and Gordon, R. F. (1952). *Brit. vet. J.*, **108**, 338.
- Davis, O. S. (1952). *Amer. J. vet. Res.*, **13**, 105.
- Eckert, E. A., Beard, O., and Beard, J. W. (1951). *Cancer Res.*, **11**, 245.
- Ellerman, V., and Bang, O. (1908). *Zbl. Bakt.*, **46**, 595. (Cited by Engelbreth-Holm, 1942).
- Emmel, M. W. (1950). *Bull. Flo. Univ. Agr. Exp. Sta.*, 470, 1.
- Engelbreth-Holm, J. (1942). *Spontaneous and Experimental Leukaemia in Animals*. Edinburgh: Oliver & Boyd.
- Feldman, W. H. and Olson, C. (1948). *Diseases of Poultry* by Biester, H. E. and Schwartz, L. H., 2nd Ed. Iowa State College Press; Ames, Iowa.
- Furth, J. (1929). *Proc. Soc. exp. Biol. N.Y.*, **27**, 155; (1933). *J. exp. Med.*, **58**, 253.
- Gottschalk, R. G. (1943). *Cancer Res.*, **3**, 649; (1946). *Ibid.*, **6**, 270.
- Gross, L. (1950). *Proc. Soc. exp. Biol. N.Y.*, **73**, 246.
- Jarmai, K. (1930). *Arch. wiss. prakt. Tierheilk.*, **62**, 113.
- Jungherr, E. (1948). In *Diseases of Poultry* by Biester, H. E. and Schwartz, L. H., 2nd. Ed. Iowa State College Press; Ames, Iowa.
- Jungherr, E., Durant, A. J., and Lee, C. D. (1941). *Amer. J. vet. Res.*, **2**, 116.
- Oberling, Ch., and Guerin, M. (1934). *Bull. Ass. franc Cancer*, **23**, 38; Olson, C. jnr. (1941). *Cancer Res.*, **1**, 384; (1945a). *Cornell Vet.*, **35**, 220. (1945b). *Ibid.*, **35**, 308; (1945c). *Amer. J. vet. Res.*, **6**, 103; (1946) *Cornell Vet.* **36**, 41; (1947). *Ibid.*, **37**, 231.
- Pentimalli, F. (1940). *Accad. Lincei*, **1**, 395; (1941). *Cancer Res.*, **1**, 69 (Abstract).
- Sharpless, G. R., Olson, J. J., Little, P. O., and Davies, M. D. (1950). *Poult. Sci.*, **29**, 818.
- Shear, H. Imagawa, D. T., Syverton, J. T., and Bittner, J. J. (1951). *Proc. Soc. exp. Biol. N.Y.*, **78**, 281.
- Wolfe, H. R., and Dilkes, E. (1947). *J. Immunol.*, **58**, 245.

(Received for publication, November 29th, 1952.)

PLATE I



LEGENDS TO PLATE

- Fig. 1. Extensive tumour cell involvement of the liver of a chick inoculated with tumour strain Rpl. 12. Magnification $\times 325$.
- Fig. 2. Section of normal chick liver. Magnification $\times 325$.
- Fig. 3. Section of pectoral tumour from a young chick inoculated with tumour strain Rpl. 19. Tumour cells are seen to form the wall of a blood vessel, indicated by arrow. Magnification $\times 325$.
- Fig. 4. Section of the pectoral muscle of a bird inoculated as an adult with tumour strain Rpl. 19, showing diffuse infiltration of pectoral muscle with tumour cells. Magnification $\times 115$.
- Fig. 5. Nodular infiltration with tumour cells of pectoral muscle. Magnification $\times 65$.
- Fig. 6. Gross section of a pectoral tumour in a bird dying following inoculation at 56 days, of tumour strain Rpl. 12.

STUDIES ON THE AVIAN LEUKOSIS COMPLEX*

3. A NOTE ON THE MAINTENANCE OF AVIAN
LYMPHOID TUMOUR STRAINS

By

C. LE Q. DARCEL†

Animal Health Trust, Poultry Research Station, Houghton, Hunts.

INTRODUCTION

In the previous paper (Darcel, 1953) the behaviour of three transplantable avian lymphoid tumours in twenty-five serial passages was studied. It was stated that the mortality in the experimental birds was 95 per cent. From this it might be thought that the maintenance of these strains would not present any serious difficulties. Although in the series of experiments discussed, loss of activity was not experienced, suspensions prepared from an apparently tumour-involved liver sometimes failed to produce tumours in inoculated chicks. If the passage had not been duplicated these strains could not have been maintained.

Burmester, Brandly and Prickett (1944), and Burmester (1950), have shown that material from these avian lymphoid tumour strains remains active for considerable periods when stored *en masse*, in sealed pyrex tubes, at temperatures below -40°C . Low temperature storage, over long periods, obviates some of the disadvantages of serial passage, namely (1) the wastage of experimental animals; (2) the danger of losing the strains through failure of a suspension to produce tumour takes; (3) the danger of introducing other infectious agents, during serial passage from chick to chick.

There are obvious technical advantages in being able to store small aliquots of tumour material as suspension. Gye, Begg, Mann and Craigie (1949) and Craigie (1949a, 1949b) observed that the mouse of sarcoma C₃H can be stored successfully in suspension at -70°C for long periods. Darcel (1952a) obtained similar results with avian lymphoid tumour strains. The observations of Craigie (1949a, 1949b) that the incorporation of dextrose in the suspending media, favoured the survival of the maximum tumour producing activity, was also confirmed for these avian lymphoid tumours.

Certain workers have claimed that differences in the rates of freezing and thawing, affect the activity of tumour material (Lepine, Barski and Réinié, 1951). In his experiments on the preservation of activity of avian lymphoid tumours at low temperatures, Burmester (1950) also found that the activity was affected by the rapidity with which the material was frozen and subsequently thawed.

* These studies, which formed part of a thesis submitted for the Ph.D. degree of the University of London, were supported by a grant from the A.R.C.

† Present address: Imperial Cancer Research Fund, Burton Hole Lane, Mill Hill, London, N.W.7.

One of the possibilities of the long-term storage of tumour material as suspension is that it might offer a means whereby tumour material of predetermined activity could be used in experiments, which could be repeated at will. In this connection Craigie (1949b) has pointed out that "such a variable dependance on the rate of freezing and perhaps that of thawing does not exactly recommend preservation by freezing as a reliable practical method."

In the present paper experiments are outlined in which this aspect was examined. In all but one case, ampoules were tested before and at two different intervals after storage. In the remaining experiments two ampoules of the same suspension were tested after being stored for the same period of time. Differences in room temperature and other unrecognised variables must affect the rate of thawing. If the rates of thawing and freezing, under these conditions, exerted any significant effect on the activity of stored material, then the ampoules tested at the two intervals would show different levels of tumour producing activity. The results of the experiments to be described show that this did not occur.

METHODS

The apparatus used for the storage of tumour material has already been described by Darcel (1952a). They consisted of: (a) an electric refrigerator maintained at temperatures of below -70°C , with the aid of CO_2 ice (subsequently referred to as refrigeration A); (b) an insulated box maintained at a temperature of below -70°C , with CO_2 ice (subsequently referred to as refrigeration B); (c) a double vacuum flask containing crushed CO_2 ice in the small inner compartment (1 quart capacity) and larger outer flask (1 gallon capacity). Ampoules to be stored were placed in the inner flask. The whole unit was placed in a refrigerator running at -25°C (subsequently referred to as refrigeration C). In these investigations sterile 3 ml. glass ampoules, sealed by heat after approximately 1.5 ml. suspension has been pipetted into them, have been used. The ampoules have been placed directly, without pre-cooling, into the refrigerator or CO_2 ice in the case of (c). The breed of chicks and methods used have been those outlined in the previous paper with the exception that, suspensions have been filtered through gauze and sintered AG74 $\times 0$ glass filters prior to inoculation and storage. Thawing was accomplished by removing ampoules from the refrigerator and exposing them to room temperature.

In view of the findings of Gottschalk (1943, 1945), Craigie (1949a), Eckert, Beard and Beard (1951), and Darcel (1952b), the mean latent period has been used as a criterion of activity.

RESULTS

The results of these experiments which are summarized in Table 1 show that with the possible exception of batch T428/407 there were

TABLE I

THE EFFECT OF STORAGE AT LOW TEMPERATURES ON THE ACTIVITY OF TUMOUR SUSPENSIONS

Batch T—	Tumour Material				Refrigeration		Results of Inoculation			
	Strain	Source	Suspending fluid	Concen- tration of sus- pension	Meth- od	Time stored in days	No. of chicks inocu- lated	Total No. of tumours develop- ing	No. of tumours palpa- ted	Mean time in days of latent period
414/712	12	Liver	(a) Saline	1/10	B	0	4	4	4	6.00 ± 0
			(b) "	1/10		24	5	0	0	—
			(c) "	1/10		183	3	1	1	14.00 ± 0
428/407	19	Pector- al Tu- mours	(a) Saline	1/5	B	0	8	8	8	6.13 ± 0.23
			(b) "	1/5		12	5	4	4	17.50 ± 0.50
			(c) "	1/5		160	4	0	0	—
			(d) 10% Dextrose	1/5		0	7	7	6	6.14 ± 0.26
			(e) " "	1/5		12	5	5	5	10.66 ± 0.77
			(f) " "	1/5		160	6	6	6	10.00 ± 0.37
			(g) Mince	—		12	7	7	7	11.43 ± 0.20
			(h) "	—		160	6	6	6	11.33 ± 0.33
485/689 627	19	Liver	(a) 6% Dextrose	1/5	A	0	4	4	4	5.25 ± 0.25
			(b) " "	1/5		94	6	6	6	5.83 ± 0.17
			(c) " "	1/5		94	5	5	5	5.40 ± 0.24
484/604	16	Liver	(a) Saline	1/10	B	0	4	4	4	6.50 ± 0.78
			(b) "	1/10		9	3	0	0	—
			(c) "	1/10		95	4	0	0	—
			(d) 6% Dextrose	1/10		0	5	5	5	6.60 ± 0.30
			(e) " "	1/10		9	4	4	4	11.00 ± 0.97
			(f) " "	1/10		95	3	3	3	11.33 ± 0.89
533/ 9019	16	Pector- al Tu- mour	(a) 6% Dextrose	1/10	C	0	8	8	8	5.50 ± 0.19
			(b) " "	1/10		3	6	6	6	7.33 ± 0.33
			(c) " "	1/10		6	8	8	8	6.63 ± 0.18
554/113	16	Liver	(a) Saline	1/10	C	0	7	7	7	6.57 ± 0.53
			(b) "	1/10		7	4	4	4	11.25 ± 0.25
			(c) "	1/10		33	4	4	4	10.50 ± 0.50

no significant differences in activity when tested at different intervals of time. Five chicks were inoculated with saline suspensions from T_{428/407} after it had been stored for 12 days. Tumours did not appear until 17.50 ± 0.50 days after inoculation. Four chicks inoculated with the same material which had been stored for 160 days failed to develop tumours. These observations might suggest that the latter material was less active, but our previous experience with these tumours leads us to believe that the latent period of 17.50 ± 0.50 days with the material stored 12 days infers minimum tumour producing doses having been inoculated. Thus the difference in the activity of the 12 day material and the loss of activity by the 160 day material is so slight as to be of doubtful significance. The

reverse situation will be seen to have followed the inoculation of batch 414/712.

The observation by Darcel (1952b) that dextrose had a protective effect on the preservation of the activity of these avian lymphoid tumours at low temperatures has been confirmed by the present experiments. In experiment T484/604 the saline suspension of Rpl.16 tested before refrigeration produced tumours in 6.50 ± 0.78 days. The same material no longer produced tumours when stored for 9 and 95 days, but the dextrose suspension stored for similar periods produced tumours with latent periods of 11.00 ± 0.97 days and 11.33 ± 0.89 days respectively.

A comparison was made of the activity after storage of saline and dextrose suspensions and undiluted mince from pectoral tumour T428/407. The tumour mince after thawing was diluted prior to inoculation to approximately the same concentration of the suspensions. It will be seen that the activity of material stored in dextrose (latent period—10 to 11 days) was greater than that stored as a saline suspension (latent period— 17.50 ± 0.50 days), whilst the material stored as tumour mince was intermediate in activity (latent period 11.0 ± 1.5 days).

DISCUSSION

Considerable research into the ability of normal and neoplastic living tissues and also living organisms to survive low temperatures has been carried out in the last few years (Passey and Dmochowski, 1950; Lepine, Barski and Réinié, 1951). The factors underlying the survival of tumour cells at low temperatures and the means by which the incorporation of dextrose contributes to their resistance are undoubtedly complex. They have been discussed by Craigie (1949b); Craigie, Lind, Hayward and Begg (1951), and Craigie (1952). The protective effect of dextrose has been very marked in the present experiments and those described in a previous paper (Darcel, 1952a). For these reasons it is suggested that, pending the discovery of even more favourable suspending media, aqueous solutions containing 6 or 10 per cent. dextrose be used for the routine maintenance of avian lymphoid tumours. Both concentrations were used in these experiments with equally favourable results.

The variation in the rates of freezing and thawing that must occur, in the conditions described in these experiments, did not significantly affect the activity of tumour material when tested on two separate occasions after storage over periods of several weeks. This suggests that storage at low temperatures offers a means by which tumour suspensions of predetermined activity can be used in successive experiments, and opens up considerable possibilities in experimental research.

A drop in the activity of tumour material which occurs during the process of freezing, even in suspensions made in glucose, suggests that the death of a considerable number of tumour cells has occurred

even under these more favourable conditions. It emphasises the need for the further study of the effects of freezing on living cells and the means by which injurious effects can be avoided.

SUMMARY

A fall in the tumour producing activity of suspensions of avian lymphoid tumours occurs after freezing to -70°C and subsequent thawing.

Dextrose exerts a protective effect in that it considerably reduces the fall in activity.

Suspensions tested at two separate intervals after storage at -70°C have shown no significant differences in activity.

The suggestion is made that the storage of tumour suspension at -70°C offers a method by which material with known activity can be employed in experiments.

ACKNOWLEDGMENTS

The writer takes this opportunity to express his grateful thanks to Dr. J. Craigie, Scientific Director of the Imperial Cancer Research Fund, and Mr. S. F. Hodgman, Director of the Animal Health Trust's Canine Research Station, for the refrigeration facilities placed at his disposal.

REFERENCES

- Burmester, B. R. (1950). *Cancer Res.*, **10**, 708.
Burmester, B. R., Brandley, C. A., and Prickett, C. O. (1944). *Proc. Soc. exp. Biol. N.Y.*, **55**, 203.
Craigie, J. (1949a). *Brit. med. J.*, *ii*, 1485; (1949b). *Brit. J. Cancer*, **3**, 268; (1952). *J. Path. Bact.*, **64**, 251.
Craigie, J., Lind, P. E., Hayward, M. E., and Begg, A. M. (1951). *Ibid.*, **63**, 177.
Darcel, C. le Q. (1952a). *Brit. J. Cancer*. (In press); (1952b). *Thesis, London*; (1953). *J. comp. Path.*, **63**, 112.
Eckert, E. A., Beard, D., and Beard, J. W. (1951). *Cancer Res.*, **11**, 245.
Gottschalk, R. G. (1943). *Ibid.*, **3**, 649; (1946). *Ibid.*, **6**, 270.
Gye, W. E., Begg, A. M., Mann, E., and Craigie, J. (1949). *Brit. J. Cancer*, **3**, 259.
Lepine, P., Barski, G., and Réinié, L. (1951). *Ann. Inst. Pasteur*, **80**, 571.
Passey, R. D., and Dmochowski, L. (1950). *Brit. med. J.*, *ii*, 1129.

(Received for publication, November 29th, 1952.)

AN ENRICHMENT BROTH AND DIFFERENTIAL MEDIUM FOR THE ISOLATION OF MASTITIS ORGANISMS FROM THE MILK OF DISTANT HERDS

By

A. G. INNES

Co-operative Wholesale Society Ltd., Research Department, Manchester

INTRODUCTION

For the isolation of organisms from the milk of cows suspected of mastitis, examination of samples as soon as possible after milking is generally considered desirable. In this laboratory, which serves creameries situated over a large area, this is not always possible.

At one creamery situated some distance from the laboratory concern was felt about the possible incidence of mastitis in herds, the milk from which was used for cheesemaking. There were no provisions at the creamery for detailed bacteriological investigations and milk samples sent to this laboratory were often many hours old when received. A method was required which would facilitate the isolation of streptococci and *Staphylococcus aureus* from samples which might be up to 48 hours old when received. In considering methods of examination to be used in the present investigation it had to be borne in mind that the milk samples would in some cases be taken by the farmer himself under the supervision of the Farms' Inspector from the creamery. As the milks might not be obtained under aseptic conditions in every case an enrichment medium which would tend to suppress contaminating organisms would be of advantage. The amount of growth of any contaminants present would no doubt be determined by the length of time the samples were in transit between the creamery and this laboratory. In no case was it expected that the time would be less than 12 hours and in extreme cases might be as much as 48 hours. The bottles in which the samples were taken would have to stand up well to repeated autoclaving and rail travel. Numerous selective media and enrichment methods were considered with a view to instituting a satisfactory method of examination of milk samples for mastitis organisms.

Neave, Taylor and Lawes (1949) have described a combined sampling and plating method for diagnosing intra-mammary infections. Farmers were supplied with rectangular bottles one surface of which was covered by a layer of aesculin blood agar. The milk was sampled direct on to the agar, the excess drained off and the bottle posted to the laboratory. Of the many selective media and enrichment methods suggested by other workers (Chodkowski and Lancaster, 1949; Davis, 1941; Davis and McClemon, 1939; Davis, McClemon and Rogers, 1939; Edwards, 1933, 1938; Little, 1940; Mattick, Shattock and Jacob, 1941; Williams

and Hirsch, 1950), none seemed to satisfy entirely our particular requirements. The method suggested by Neave, *et al.* (1949) was not entirely satisfactory for during transit the agar slopes tended to break up making it difficult to pick off suspected colonies and an enrichment broth technique was regarded as the most suitable for our purpose.

PRELIMINARY OBSERVATIONS

Preliminary observations were made using the following cultures. *Strept. agalactiae* N.I.R.D. Nos. 943 and 944 kindly supplied by F. K. Neave. *Strept. dysgalactiae* N.C.T.C. No. 4671. *Strept. uberis* N.C.T.C. No. 3858. *Strept. faecalis*, *Strept. lactis* and *Staph. aureus* from stock cultures held in this laboratory. The *Strept. faecalis* was isolated from a sample of fish paste suspected of causing food poisoning; the *Strept. lactis* from a routine milk sample; and the *Staph. aureus* from a sample of milk suspected of containing mastitis organisms. Wide-mouthed 4 oz. "Cream of Magnesia" bottles fitted with a screw cap with rubber liner and marked at 75 ml were found convenient to hold the enrichment broth and milk, and were used throughout the investigation.

In the first instance laboratory trials were made by inoculating a loopful of the mastitis streptococci, incubated in broth for 6 hours at 37°C, into bottles containing sterile milk with 1:100 glucose, 1:40,000 sodium azide and 1:20,000 bromocresol purple. In an attempt to reproduce artificially the conditions which the samples might have to undergo during their transit from the creamery to this laboratory, the bottles were seeded with pure cultures of streptococci and treated as follows:

(a) Immediately after inoculation incubated for 24 hours at 37°C, and then streaked from the bottles on to the surface of 5 per cent horse blood agar plates.

(b) Stored at 21°C for 24 hours followed by incubation for 24 hours at 37°C before streaking on to 5 per cent horse blood agar plates.

(c) Refrigerated at 4°C for 24 hours, stored at 21°C for 24 hours and then incubated for 24 hours at 37°C, before streaking on to 5 per cent horse blood agar plates.

In all cases large numbers of the organisms were recovered from the broths indicating that no inhibition had occurred.

Quarter samples from cows suspected of having mastitis were tested by this method. Evening milk was sampled directly into the bottles to the 75 ml mark and containing as final concentrations, glucose 1:100, sodium azide 1:40,000 and bromocresol purple 1:20,000. They were stored at atmospheric temperature until received in the laboratory next morning, when they were incubated for 24 hours at 37°C. Streaks were then made on 5 per cent horse

blood agar plates, from which, after incubation, many colonies had to be picked off for examination and a large proportion of these proved not to be mastitis organisms. In view of the criticism by Mattick *et al.* (1941) of Edwards' medium and of the azide broth enrichment method it was decided to investigate other media in an attempt to find one which would eliminate false positives without suppressing mastitis streptococci and which would give distinctive colonies on streak culture. Of the various media tried excellent growths of the test organisms were obtained in an agar prepared from brain-heart infusion broth (Difco) to which was added aesculin, ferric chloride, yeast autolysate, litmus solution and crystal violet in proportions given later in the paper. The litmus solution and yeast autolysate were prepared according to the methods given by Davis (1935). The medium was later modified by omitting the litmus solution, which was found to serve no useful purpose and by doubling the aesculin concentration, thereby obtaining a better differentiation between the aesculin fermenters and non-fermenters. Later the addition of 1:40,000 sodium hippurate to the medium was found to give a better differentiation between *Strept. agalactiae* and *Strept. dysgalactiae*, colonies of these organisms reaching a diameter of 2.5 to 3 mm. after 48 hours. *Strept. uberis* was not readily differentiated from small colonies of *Strept. lactis* and *Strept. faecalis*, and a primary enrichment medium was sought which would encourage proliferation of *Strept. uberis* while suppressing non-mastitis streptococci.

Ludlam (1949) described a selective medium containing mannitol, dipotassium hydrogen phosphate, lithium chloride and potassium tellurite, for the isolation of *Staph. aureus* from heavily contaminated material. A modified broth was therefore made containing glucose, lithium chloride, potassium tellurite and bromocresol purple which, with sterile milk added gave good enrichment of *Strept. agalactiae*, *dysgalactiae* and *uberis* and *Staph. aureus* (see Page 139). After passage through this enrichment broth *Strept. uberis* gave colonies on brain-heart aesculin hippurate agar which could be readily differentiated from those of *Strept. faecalis* and *Strept. lactis*. These colonies were entire edged and umbonate with a raised margin having a "motor car wheel" appearance. This enrichment medium supported the growth of small numbers of *Strept. agalactiae*. After incubation for 48 hours an acid/clot was produced and a good growth of *Strept. agalactiae* was obtained from all the tubes. No growth of *Staph. aureus* occurred on the brain-heart aesculin hippurate agar, but this organism was recovered from the enrichment medium when transferred to Ludlam's agar. When compared with blood agar it was found that Ludlam's agar gave 5 positives out of 24 samples whereas blood agar gave none. This observation together with the fact that excessive growth of contaminating organisms occurred on blood agar owing to the age of the samples when received in the laboratory, led to the exclusive use of Ludlam's agar for the isolation of *Staph. aureus* from milk samples.

FINAL METHOD

Media

(a) *Glucose, lithium chloride, potassium tellurite, bromocresol purple broth.*

Screw-capped bottles as previously described and containing
 37.5% w/v glucose solution 2 ml
 30% w/v aqueous solution of lithium chloride .. . 1 ml
 1% w/v alcoholic solution of bromocresol purple .. 0.35 ml
 are autoclaved at 8 to 10 lbs. pressure for 20 to 30 minutes.

After cooling 1.5 ml of a 1:400 sterile aqueous solution of potassium tellurite is added with aseptic precautions. The bottles must not be heated further after the addition of the potassium tellurite.

The final concentrations after the addition of the milk to the 75 ml mark are glucose 1:100, lithium chloride 1:250, bromocresol purple 1:21,500, potassium tellurite 1:20,000.

(b) *Brain heart aesculin hippurate agar*

Brain heart infusion (Difco)	3.7 g.
Agar	1.5 g.
Aesculin	0.2 g.
Yeast autolysate	1.0 ml
5.8% ferric chloride, aqueous solution ..	0.1 ml
0.1% crystal violet, aqueous solution ..	0.2 ml
0.25% sodium hippurate, aqueous solution ..	1.0 ml
Distilled water	100 ml

Adjust to pH 7.4

Autoclave at 10 lbs pressure (115°C) for 20 minutes, cool and pour plates 2 to 3 mm. deep.

The procedure finally adopted in this laboratory for the examination of milk samples from distant herds for mastitis organisms was as follows :

The milk from suspected cows was sampled directly into the screw capped bottles, containing 4.85 ml of the glucose, lithium chloride, potassium tellurite bromocresol purple medium, to reach the 75 ml mark. The bottles were then despatched by rail on the day the samples were taken, or else refrigerated overnight at the creamery and despatched the following day. The samples were not artificially cooled during rail transit. On arrival at this laboratory the bottles were immediately incubated for 24 hours and two loopfuls then streaked on to plates of brain heart aesculin hippurate agar and Ludlam's agar. The latter were incubated for 48 hours and then examined for the presence of colonies of *Staph. aureus* which were confirmed by the coagulase test. The brain heart aesculin hippurate agar plates were incubated for 24 to 48 hours, when suspicious colonies were picked off into tubes of yeast aesculin litmus milk (Davis and Rogers, 1939) and into broth containing 1 per cent sodium hippurate. The yeast aesculin litmus milk tubes were examined visually and microscopically after 48 hours incubation ; the hippurate broth tubes were tested for hydrolysis

of the hippurate after 5 days incubation, an uninoculated tube being included as a control.

RESULTS

The examination was carried out with a hand-lens by transmitted daylight. The nature of the colonies on agar after enrichment were as follows :

Strept. agalactiae : Regular, entire, crenated or lobate, raised with a brownish purple diffuse centre fading to a pale periphery, 1 to 2.5 mm. diameter. (Fig. 1).

Strept. dysgalactiae : Regular, entire, convex, purple with a narrow periphery, 1 to 2.5 mm. diameter. The colony can be easily pushed along the surface of the medium. (Fig. 2).

Strept. uberis : Entire, umbonate with raised margin, light brown with slightly dark brown centre, 0.5 to 1 mm. diameter. (Fig. 3).

Strept. faecalis : Regular, entire, conical, dark brown centre fading to a light brown periphery, 0.5 to 1.5 mm. diameter. (Fig. 4).

Strept. lactis : Regular, entire, raised, dark brown centre with successive lighter bands to a pale periphery, 1 to 2.5 mm. diameter. (Fig. 5).

The appearance of *Strept. lactis* and *Strept. faecalis* is included for purposes of comparison.

Strept. agalactiae and *Strept. dysgalactiae* do not produce darkening in the medium and can be differentiated at sight from aesculin fermenting organisms which produce a brown zone round the colonies.

Strept. uberis is the only organism encountered which gives the typical "motor car tyre" appearance and this appearance has only been noted after the organism has been enriched in the glucose, lithium chloride, potassium tellurite, bromocresol purple broth.

The method described afforded this laboratory an opportunity of examining milk samples from herds situated at some distance. As *Strept. agalactiae* was isolated from samples which were 40 hours old when received in the laboratory no concern was felt about the age of the samples. Provided reasonable aseptic conditions were maintained at the time of sampling there was no interference from contaminating organisms and in a great many cases pure growths of *Strept. agalactiae* were obtained.

As a comparison with azide broth enrichment, out of 7 known positive samples azide broth enrichment gave 3 positives while glucose, lithium chloride, potassium tellurite broth enrichment gave 7 positives.

The method has been used in this laboratory for a period of twelve months and of 184 samples examined, 31 (16.84 per cent) were found to contain mastitis streptococci—

Strept. agalactiae 25 (13.58 per cent), *Strept. dysgalactiae* 5 (2.72 per cent) and *Strept. uberis* 1 (0.54 per cent) ; 22 (11.96 per cent) were found to contain *Staph. aureus*. The positive *Strept. agalactiae* isolations

included 4 samples from cows known to have been positive and which had been treated with penicillin four days previous to taking the milk samples. They also included samples from 6 cows which had been treated with Protogan under the supervision of Bangor University : 3 were found to be positive for *Strept. agalactiae*. Thirty-six cows were tested with a view to the suitability of their milk for use as starter cultures ; of these, 2 were found to be infected with *Strept. agalactiae*, these results being included in the above.

SUMMARY

By the use of an enrichment broth and differential media mastitis streptococci and *Staph. aureus* may be isolated from the milk of distant herds. Bottles containing glucose, lithium chloride, potassium tellurite and bromocresol purple are inoculated with the milk samples. On arrival at the laboratory the bottles are incubated at 37°C for 24 hours, after which streak inoculations are made on to brain heart aesculin hippurate agar to isolate *Strept. agalactiae*, *Strept. dysgalactiae* and *Strept. uberis* and on to Ludlam's agar to isolate *Staph. aureus*. The morphology of colonies of the mastitis streptococci on brain heart aesculin hippurate agar which proved distinctive is described.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Mr. A. Glover for granting permission and facilities to carry out this work, to Mr. H. G. Coles for his help and continued interest throughout the investigations, to Mr. A. Rowlands of Astell Laboratory Service Co. Ltd., for his help in arranging the final draft of this paper, and to Mr. S. J. Bushnell for preparing the photographs. This paper is published with the permission of the Directors of the Co-operative Wholesale Society Limited, Manchester.

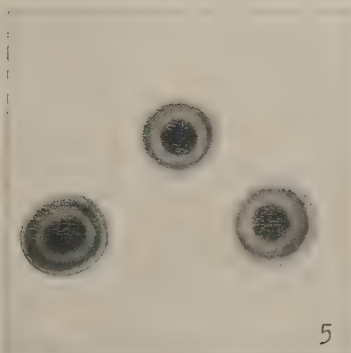
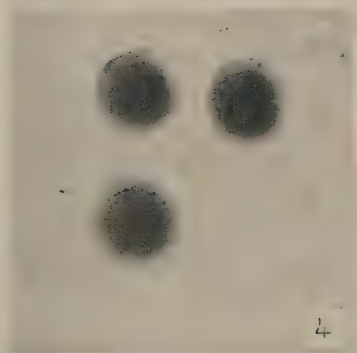
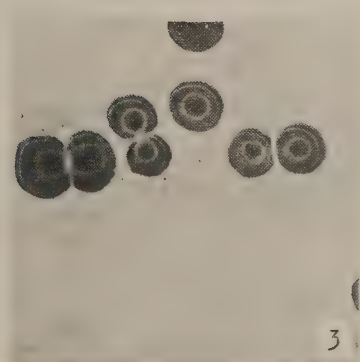
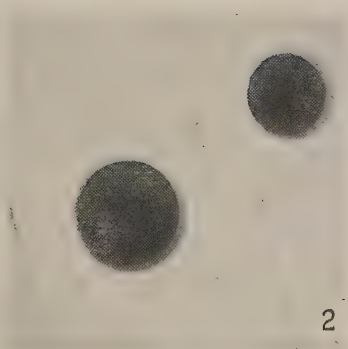
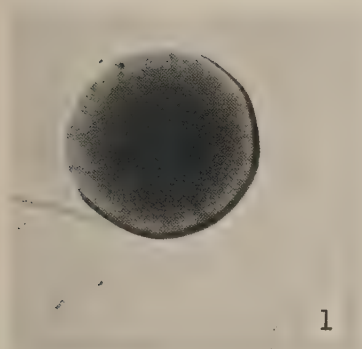
REFERENCES

- Chodkowski, A., and Lancaster, J. E. (1949). *J. comp. Path.*, **59**, 265.
 Davis, J. G. (1935). *J. dairy Res.*, **6**, 121; (1941). *Dairy Industr.* Feb. p. 38.
 Davis, J. G., and McClemon, J. (1939). *J. dairy Res.*, **10**, 74.
 Davis, J. G., McClemon, J., and Rogers, H. J. (1939). *Ibid.*, **10**, 59.
 Davis, J. G., and Rogers, H. J. (1939). *J. comp. Path.*, **52**, 132.
 Edwards, S. J. (1933). *Ibid.*, **46**, 211; (1938). *Ibid.*, **51**, 250.
 Little, R. B. (1940). *J. Amer. vet. med. Ass.*, **97**, 212.
 Ludlam, G. B. (1949). *Mon. Bull. M.O.H. and P.H.L.S.*, **8**, 15.
 Mattick, A. T. R., Shattock P. M. F., and Jacob, M. Moriera (1941). *J. dairy Res.*, **12**, 139.
 Neave, F. K., Taylor, M. E., and Lawes, L. F. (1949). *Proc. Soc. app. Bact.*, p. 34.
 Stirling, A. C. (1940). *Proc. Soc. agric. Bact.*, p. 32.
 Watts, P. S. (1940). *J. dairy Res.*, **11**, 316.
 Williams, R. E. O., and Hirsch, Ann (1950). *J. Hyg. Camb.*, **48**, 504.

[Received for publication, 10th October, 1952]

LEGENDS FOR FIGURES

- Fig. 1. *Strept. agalactiae*; after enrichment in glucose, lithium chloride, tellurite broth. 48 hours incubation at 37°C. Brain heart aesculin hippurate agar. $\times 11$.
- Fig. 2. *Strept. dysgalactiae*; after enrichment in glucose, lithium chloride, tellurite broth. 48 hours incubation at 37°C. Brain heart aesculin hippurate agar. $\times 11$.
- Fig. 3. *Strept. uberis*; after enrichment in glucose, lithium chloride, tellurite broth. 48 hours incubation at 37°C. Brain heart aesculin hippurate agar. $\times 11$.
- Fig. 4. *Strept. faecalis*; after enrichment in glucose, lithium chloride, tellurite broth. 48 hours incubation at 37°C. Brain heart aesculin hippurate agar. $\times 11$.
- Fig. 5. *Strept. lactis*; after enrichment in glucose, lithium chloride, tellurite broth. 48 hours incubation at 37°C. Brain heart aesculin hippurate agar. $\times 11$.



OUTBREAK OF NOCARDIOSIS IN MARSUPIALS IN THE
BRISBANE BOTANICAL GARDENS

By

R. TUCKER and R. MILLAR

*Faculty of Veterinary Science, University of Queensland,
Brisbane, Australia.*

INTRODUCTION

The diagnosis of mycotic disease in mammals is handicapped by difficulties in classification and nomenclature of the causative organisms. The additional obstacles created by the similarity of the clinical syndromes in most mycotic diseases, as well as difficulties in obtaining cultures of the slow-growing and facultative fungi, often make the exact diagnosis rather uncertain. The role of streptothrix, as saprophytes as well as pathogens, confuses the clinical diagnosis.

The species classification is based on either morphological characteristics, e.g., *Nocardia asteroides* and *Actinomyces asteroides* (Eppinger, 1891; Garrod, 1952) or on the pathogenic properties of mycotic organisms for certain species of animals, as *Actinomyces*, *Nocardia* or *Streptothrix canis* (Chalmers and Christopherson, 1916-1917), *bovis* (Reategui, 1947), *hominis* (Fullerton, 1913), *macropodidarum* (Le Souef and Seddon, 1929) and there are further classifications according to their oxygen requirements or their response to acid-fast stains (Wilson and Miles, 1948).

As the pathogenicity of the mycotic organisms in certain conditions may involve more than one host (Lesbouyries, 1942), the problem of nocardiosis is important for comparative as well as preventive medicine. In Australia fungoid diseases have been reported in marsupials by Le Souef and Seddon (1929), in dogs and cattle by Millar (unpublished observations), in horses (Burns and Simmons, 1952), in sheep (Henry, 1952) and in man (Goldsworthy, 1937).

Actinomycosis in the dog has been recorded in Egypt (Mansi, 1952), England (McGaughey, Bateman and McKenzie, 1951) and Scotland (Ginsberg and Little, 1948). Miles (1951) described actinomycosis in the antelope (*Aurebia kenyaë*) in the London Zoo. In Peru its occurrence has been reported in sheep by Reategui (1947), and in France it has been recorded by Carpentier (1939) in horses, and Lesbouyries (1952) in sheep.

There are various reports of the disease in man and many types of animals in America (see review by Tucker and Hirsch, 1949).

CLINICAL HISTORIES

The marsupials in the Brisbane Botanical Gardens are kept in a large pen and with them are deer, emus, and water-birds. All are artificially fed. Over a two-month period during 1951 to 1952 we were able to investigate the cause of death of four out of eight wallabies and kangaroos,

Case 1. A young male kangaroo was admitted with ulcerative gingivitis at the level of the upper incisors and the anterior nares. It also showed marked swelling of the left face involving the masseter muscles and parotid glands. A purulent discharge from the left nostril and eye was present. Swabs from the lesions in the nose, eye and gums were taken for bacteriological investigations. These revealed the *Nocardia* spp. A good growth in the form of a granular deposit was obtained in serum broth cultures incubated anaerobically for three days. The granules consisted of colonies taking the form of gram-positive filamentous branching mycelia. After six days on blood agar, small colonies (0.5 mm.), coral pink in colour, were apparent. They were non-haemolytic, aerobic, irregular in shape and adherent to medium. These organisms gave no fermentation reactions.

The animal received 200,000 units of penicillin daily. Recovery took place after ten days and the patient was sent back to the Gardens. Four weeks later the animal relapsed and was returned to the clinic *in extremis*. Death occurred shortly after.

Case 2. A female wallaby was reported ill in the morning and died in the afternoon. There was marked swelling on the face and purulent superficial lesions were seen, similar to those previously described. Material was taken from the submandibular region, lower lips and nostril. An organism similar to that reported in Case 1 was isolated and diagnosed bacteriologically as *Nocardia* spp.

Case 3. A young male kangaroo in good condition. On admission the only symptoms were swelling of the face and enlargement of the submandibular lymphatic glands. The glands were removed for biopsy. The results of the bacteriological investigation were negative, but the lesion appeared to be a chronic lymphadenitis with fibrosis and decomposition of cortical and medullary tissues. There were many small and a few large necrotic foci, surrounded by capillaries, neutrophils and macrophages. Some basophilic filamentous organisms were seen. The blood in the larger vessels in the section showed a great excess of neutrophile leucocytes. In a few days the animal became very weak and cachetic. Its face and lower jaw became hard, swollen and painful and it refused to eat. Treatment was considered to be of no use and it was destroyed *in extremis*. An autopsy revealed no internal lesions in any organs. A bacteriological examination of the head failed to reveal the presence of nocardiosis.

Case 4. A female wallaby was admitted in a state of collapse and died shortly afterwards. Swabs were taken from abscesses in the submandibular region, from the nasal cavity and from a swelling of the lower lip. From these *Nocardia* spp. were isolated on blood agar and in serum broth, as previously described. Colonies of *Nocardia* were present on sheep's blood agar after six days' incubation at 37°C. Autopsy revealed changes in the kidneys, fatty changes in the liver, congestion of the lung and oedema over the parietal region of the myocardium.

DISCUSSION

The world-wide distribution of mycotic fungi as saprophytes and pathogens in man and animals has been reviewed. The cases discussed here, which were part of an outbreak of nocardiosis, show several outstanding features.

The outbreak affected a number of animals, but the cases were confined to marsupials only, although other herbivores were present and living under similar conditions. The type of food and the proximity in which these animals lived and fed may have been the primary factor in the epidemic. Generally actinomycosis has been reported as a sub-acute or chronic disease, but in this outbreak it seemed to assume an acute phase. Rosebury (1944) in describing the relationship between salivary calculus in man and actinomycosis states that it is a sub-acute or chronic infection of connective tissue primarily by *Actinomycosis bovis* or *israeli*, these names being synonymous. However, Thompson (1950), in agreement with other workers, says that *Actinomycosis bovis* and *A. israeli* are two distinct anaerobic species. As distinct from Rosebury's observations we noted the primary lesion in lymphatic glands in one suspected though unconfirmed case and this was followed by secondary swelling of the connective tissues three or four days later.

There seems little doubt that the early stages may be asymptomatic or difficult to detect in wild animals and thus the animal appears debilitated before any clinical manifestations are perceived. The secondary symptoms appear to result from cachexia and dehydration, due to faulty mastication and the inability to swallow.

Le Souef and Seddon (1929) described an epizootic in marsupials and they considered artificial feeding and captivity to be the prime factors in the spread of the disease. The authors tried various forms of treatment without success, but a vaccine seemed to be of some value as a preventive. Recent references to treatment are widespread. Tucker and Hirsch (1949) conclude that anaerobic types of actinomycotic infections appear to respond to sulphadiazine or other sulphonamides rather than to antibiotics as penicillin and streptomycin. Garrod (1952), on the other hand, states that penicillin is the standard drug in the treatment of actinomycosis although sulphonamides may have some effect. He points out, however, that the resistance of some types of actinomycosis to penicillin is marked, and says that in others the benefit of treatment may be only temporary or absent. In his experience the mean minimum inhibitory concentration of penicillin in man was 0.06 mg/ml. In vitro our strain proved penicillin sensitive to 0.25 mg/ml: on the other hand, Case No. 1 treated with penicillin appeared to respond temporarily and then relapsed.

SUMMARY

An epizootic caused by *Nocardia* spp. amongst marsupials kept in captivity is reported. There were eight fatal cases, four of which are described.

Literature is cited showing the various names and classifications of *Nocardia*, making accurate identification difficult.

All cases observed ran a rapid course following the detection of macroscopic lesions and ended in death which may have been accelerated by secondary causes such as inability to feed and drink.

In one case the primary lesion appeared to be in a lymphatic node though its exact nature was not confirmed bacteriologically.

Nocardia spp. from a marsupial appeared to be penicillin sensitive, *in vitro*; nevertheless, the response of the animal to penicillin was disappointing.

ACKNOWLEDGMENT

We wish to express our thanks to A. K. Sutherland, B.V.Sc., M.S., and G. C. Simmons, B.Sc., of the Department of Agriculture and Stock, Yeerongpilly, and J. T. Cunneen, Q.D.D., Faculty of Veterinary Science, for their help in carrying out the bacteriological and pathological work.

REFERENCES

- Baudet, E. A. R. F. (1934). *Ann. Parasit. hum. comp.*, **12**, 296.
 Burns, R. H. G. and Simmons G. C. (1952). *Aust. vet. J.*, **28**, 34.
 Carpentier, G. (1939). *Parasites et Maladies parasitaires des équides domestique*. Vigot Freres, Paris.
 Chalmers, A. J., and Christopherson. (1916-1917). *Ann. trop. Med. Parasit.*, **10**, 223.
 Eppinger, H. (1891). *Beitr. path. Anat.*, **9**, 287.
 Fullerton, A. G. R. (1913). *Lancet*, **184**, 381.
 Garrod, L. P. (1952). *Brit. med. J.*, 1263.
 Ginsburg, A. H., and Little, A. C. N. (1948). *J. Path. Bact.*, **60**, 563.
 Goldsworthy, N. E. (1937). *Ibid.* **45**, 17.
 Henry, J. N. (1952). *Agric. Gaz. N. S. W.*, **63**, 108.
 Lesbouyries, G. (1942). *Rec. Med. vet. exot.*, **118**, 193.
 Mansi, W. (1952). *Brit. vet. J.*, **108**, 14.
 McGaughey, C. A., Bateman, J. K., and McKenzie, P. A. (1951) *Ibid.*, **107**, 428.
 Millar, R. (1952). *Unpublished observations*.
 Miles, A. A. (1951). *Proc. R. Soc. Med.*, **44**, 865.
 Rabe. (1888). *after Lesbouyries* (1942).
 Rategui, R. C. (1947). *Revista Facultad Med. Vet. Lima.*, **2**, 1 and 29.
 Rosebury, T. (1944). *Bact. Rev.* **8**, 223.
 Soeuf Le A. S., and Seddon H. R. (1929). *Aust. vet. J.*, **5**, 79.
 Thompson, L. (1950). *Rep. Mayo Clinic*, **25**, 81.
 Tucker, F. C., and Hirsch E. F. (1949). *J. infect. Dis.*, **85**, 1 and 72.
 Wilson, G. S. and Miles, A. A. (1948). *Topley & Wilson's "Principles of Bacteriology and Immunity Vol. 1*, 381. Edward Arnold & Co., Lond.

(Received for publication, November 4th, 1952).

AN ANTI-TUBERCULOUS SUBSTANCE IN
TUBERCULOUS ORGANS

By

M. A. SOLTYS

Department of Veterinary Pathology, University of Liverpool

INTRODUCTION

IN the past there have been occasional reports from bacteriologists that they have failed to isolate tubercle bacilli from tuberculous organs by cultural methods or by animal inoculation, although a microscopical examination has revealed the presence of acid-fast bacilli. Various theories have been advanced to explain this phenomenon. The view has often been expressed that such organisms are dead although they have retained their staining properties. It has also been suggested that the tissues of tuberculous animals contain an inhibitory substance, but no evidence of any decisive experiments to determine this point has been found in the literature.

In a preliminary communication (Soltys, 1952) it was reported that tuberculous lymph nodes do, in fact, contain an anti-tuberculous substance. In the present paper experiments are recorded indicating that the substance is present not only in bovine tuberculous lymph nodes but also in the liver and spleen of tuberculous guinea-pigs and rabbits.

MATERIALS AND METHODS

Preparation of the inhibiting agent. Tuberculous bovine lymph nodes from natural cases of the disease or tuberculous spleens and livers from experimentally infected guinea-pigs or rabbits were reduced to a pulp and diluted in a proportion of 100 g. of pulp to 10 ml. of Sauton's medium. The suspensions, after standing at about 4°C for 7 days, were squeezed through gauze and the fluid thus obtained centrifuged until the supernatant was free from visible particles. If sterile extracts were required this supernatant fluid was filtered through a gradocol membrane (A.P.D. 750m μ). The final product had a reaction of about pH 7.2. The inhibiting principle was much reduced in activity by filtration and by heating the fluid at 55°C for half an hour.

Strains of tubercle bacilli. The bovine strain used ("L") was of average virulence. A dose of 0.0001 mg. produced extensive lesions of tuberculosis in guinea-pigs within 6 weeks of inoculation. The human strain (H37 R.v.) was also of standard virulence. Both were maintained on Lowenstein's medium.

RESULTS

Addition of organ extract to tubercle bacilli

Bottles containing 2 ml. of extract obtained from bovine, guinea-pig or rabbit tissues were seeded with 1 mg. of the bovine strain.

Previous experiments had shown that incubation at 37°C for periods of 24 hours and 48 hours were insufficient to inactivate the bacilli. The period was therefore extended to 72 hours. Two control suspensions were prepared: one contained tubercle bacilli in an extract of non-tuberculous lymph nodes, and the other a suspension of tubercle bacilli in saline. They were incubated for a similar period. Each sample was then inoculated subcutaneously into two guinea-pigs which were killed 6 weeks later. The distribution and extent of the tuberculous lesions were noted, and smears made for the presence of acid-fast bacilli.

It was found that all guinea-pigs receiving suspensions of bacilli in the tuberculous gland or organ extracts were free from visible signs of disease, whereas those inoculated with organisms exposed to the extracts of non-tuberculous organs or to saline showed extensive generalised lesions.

Similar results were obtained when the experiments were repeated with the human strain. The results are summarised in Table I.

TABLE I

THE EFFECT OF AN EXTRACT FROM TUBERCULOUS ORGANS ON
TUBERCLE BACILLI

Organisms tested	Type of extract in which tubercle bacilli were suspended	No. of bottles	No. of guinea-pigs inoculated	Post-mortem lesions six weeks after inoculation
<i>Myc.</i> <i>tuberculosis</i> Bovine type "L"	Bovine lymph nodes	4	8	None
	Guinea-pig spleen	4	8	None
	Rabbit spleen	4	8	None
	Guinea-pig liver	4	8	None
	Rabbit liver	4	8	None
	Saline (Control)	4	8	Generalised tuberculosis
<i>Myc.</i> <i>tuberculosis</i> Human type H 37 R.V.	Bovine lymph nodes	2	4	None
	Guinea-pig spleen	2	4	None
	Rabbit spleen	2	4	None
	Guinea-pig liver	2	4	None
	Rabbit liver	2	4	None
	Saline (Control)	2	4	Generalised tuberculosis

The addition of organ extract to tubercle bacilli in tissues

The organisms used in the previous experiment were derived from cultures and were therefore probably in intimate contact with the anti-substance for a period of three days. It seemed possible, however, that tubercle bacilli in the tissues of an affected animal might not be so vulnerable. A suspension of liver and spleen from a tuberculous guinea-pig showing large numbers of acid-fast bacilli was suspended in saline, added to twice the volume of extract and left at 37°C for 4 days. Four samples were inoculated into 8 guinea-pigs (one sample to two guinea-pigs); 7 weeks later none of these animals revealed lesions of tuberculosis.

Addition of serum from tuberculous animals

The result of the preceding experiments suggested that the antibacterial agent was a soluble substance. It seemed possible that it might be present in the blood serum. An experiment similar to the preceding one was accordingly performed in which serum from tuberculous bovine animals and from tuberculous guinea-pigs was used in place of the organ extract and was compared with samples from non-tuberculous animals to determine whether the former contained protective substances which would inhibit the bacilli. The results showed that animals injected with the mixture of tuberculous serum and the virulent bacilli died sooner than the controls, an indication that the samples had, if anything, an enhancing rather than an inhibitory effect on the progress of the disease.

Effect of the extract on the viability of tubercle bacilli

The next experiments were undertaken in order to ascertain whether the action of the anti-tuberculous substance on the bacilli was bacteriostatic or bactericidal in nature. A filtered extract from tuberculous bovine lymph nodes was added to bovine bacilli and the mixture left at room temperature for six days. A half of the suspension was then divided into two parts, one of which was transferred to Lowenstein's medium, while the other was inoculated into four guinea-pigs. The other half was centrifuged, the deposit washed three times with normal saline, and the sediment resuspended in saline to the original volume: this was also divided into two parts for cultural examination and for the inoculation of four guinea-pigs. The controls comprised tubercle bacilli suspended in saline also seeded on to Lowenstein's medium and inoculated into four guinea-pigs.

After four and eight weeks respectively the number of colonies on the media were counted and one half of the group of guinea-pigs killed at each period. It was found that after four weeks the unwashed treated bacilli and the washed treated bacilli failed to grow

on the media and had produced no lesions in guinea-pigs, whereas the control fluid gave rise to a large number of colonies and evoked extensive tuberculosis in the experimental animals.

After eight weeks the unwashed treated organisms still failed to give growth in vitro or to produce visible lesions in guinea-pigs. The washed treated bacilli, however, produced a few isolated colonies on the medium and a moderate infection in the guinea-pig in which there were local lesions consisting of enlargement and caseation of the inguinal, precrucial and popliteal lymph nodes. No macroscopic changes were visible in the visceral organs. The control guinea-pigs showed generalised tuberculosis.

It would appear from these results that after treatment the tubercle bacilli were living but were prevented from exerting a pathogenic effect, possibly through a protective coating of some substance in the extract which was easily removed by washing. Bacteria which had been treated in this way and then washed were still viable and pathogenic.

Sub-inoculation of the organs of guinea-pigs inoculated with treated bacilli

In the light of the preceding experiment it was assumed that treated tubercle bacilli injected into guinea-pigs, although unable to produce any lesions, were still present in the tissues in a viable form. In order to ascertain whether such organisms could regain their pathogenicity, guinea-pigs inoculated with treated bacilli were killed six weeks later. The spleens and livers were suspended separately in Sauton's medium and inoculated into fresh guinea-pigs which were palpated from time to time for evidence of enlargement of the regional lymphatic nodes. Most of these guinea-pigs developed lesions of tuberculosis within three months.

The effect of the extract on morphology

Tubercle bacilli of both the human and bovine types were treated with extract at 37°C for three days. Smears were then stained by the Ziehl-Neelsen method. It was found that the organisms were aggregated into large clumps suggesting that they had been agglutinated. The staining properties, however, were not altered. The arrangement of the bacilli, on the other hand, was quite different from that of organisms in control smears from suspensions in non-tuberculous extracts or in saline, in which the organisms were distributed either singly or in small clumps.

Sensitivity to tuberculin of guinea-pigs inoculated with treated bacilli

Six guinea-pigs, which were inoculated with treated tubercle bacilli, failed to show any enlargement of the local lymphatic nodes. Six weeks later they were inoculated intradermally with 0.1 ml. of Weybridge mammalian P.P.D. tuberculin diluted 1/10. The same dilute tuberculin was injected into guinea-pigs which had received

bacilli in non-tuberculous extracts or in saline. There were no reactions at 24 or 48 hours in the first group, whereas both sets of controls gave positive reactions.

DISCUSSION

A substance capable of suppressing the pathogenic effect of tubercle bacilli of the human and bovine types has been demonstrated in bovine tuberculous lymph nodes (Soltys, 1952). The experiments described in this paper have shown that this anti-tuberculous substance is present not only in bovine nodes, but also in the spleen and liver of tuberculous guinea-pigs and rabbits. Attempts to separate a similar substance from the corresponding tissues of non-tuberculous animals were unsuccessful.

Sufficient experiments have not been undertaken to determine whether this anti-tuberculous substance is a form of immune body or whether it is analogous to spermine. Dubos (1951) noted that the extraction of animal tissues with water and ethanol at a low pH range yielded substances which were active against tubercle bacilli *in vitro*. In a subsequent paper by Hirsch and Dubos (1952) the inhibitory substance was identified as spermine, a substance which is widely distributed in animal tissues. An analysis of diseased organs showed that in general the concentration of spermine was higher in cases of tuberculous pneumonia and carcinoma. Since spermine appears to possess a bactericidal action on tubercle bacilli, it is unlikely that the antituberculous substance isolated from tuberculous lymph nodes which is only bacteriostatic, is identical with this chemical.

Since this substance is only liberated from the organs when the tuberculous tissue is reduced to a pulp and left for several days in the presence of Sauton's medium, it is possible that it is an anti-bacterial agent intimately linked with the tissue cells which can be freed only by maceration at a low temperature. The fact that washing treated bacilli with saline restores their pathogenic activity, and that the extract tends to aggregate the bacilli into large clumps reminiscent of an agglutination reaction, suggests that the substance present in tuberculous tissues is some form of antibody.

SUMMARY

A substance which inhibits tubercle bacilli has been demonstrated in bovine tuberculous lymph nodes and in the spleen and liver of tuberculous guinea-pigs and rabbits.

Tubercle bacilli treated with these extracts for four days failed to produce lesions in guinea-pigs and failed to render the animals sensitive to tuberculin.

The organisms were not killed, however, since sub-inoculations of spleen and liver from these animals produced local lesions in three months.

If treated bacilli were washed with saline the protective action of the extract was removed.

REFERENCES

- Dubos, R. J. (1951). *Amer. Rev. Tuberc.*, **63**, 110.
Hirsch, J. G. and Dubos, R. J. (1952). *J. exp. Med.*, **95**, 191.
Soltys, M. A. (1952). *Nature, London*, **169**, 1095.

(Received for publication, November 19th, 1952)

TERMINAL OR REGIONAL ILEITIS IN PIGS

By

H. I. FIELD,* D. BUNTAIN* and A. R. JENNINGS

Department of Animal Pathology, Cambridge University

INTRODUCTION

IN 1951 Emsbo reported the results of his studies on terminal or regional ileitis in pigs, a condition which he stated had been noted by meat inspectors for a long time. He drew attention to the close similarity between this condition and human ileitis, commonly referred to as Crohn's disease.

The condition in pigs is characterised by thickening of the ileum, most commonly in the terminal portion, so that it comes to resemble a thick-walled and rigid rubber or garden hose. In some cases the thickening is followed by ulceration and local peritonitis, while in others perforation of the ileum occurs, with escape of intestinal contents, leading to diffuse peritonitis and death.

Emsbo found that the length of the ileum involved in his cases varied from 25 to 160 cm., with an average of 75 cm. He examined 58 cases, 51 of which were detected in slaughterhouses, mostly in bacon pigs between 7 and 8 months of age. Eight of the 58 cases showed perforation of the ileum and diffuse peritonitis (14 per cent). Of 693 pigs examined during 1949 at a bacon factory at Ortved, on Zetland, 9 had ileitis and diffuse peritonitis (1.3 per cent).

The condition often affects several members of a litter and in an outbreak he investigated in September, 1950, four of a litter of 10 pigs died from diffuse peritonitis following perforation of the thickened ileum, after a short illness characterised by sudden loss of appetite, indolence, weakness of the hindquarters, a wobbling gait and repeated vomiting.

Attempts by Emsbo to demonstrate an infectious agent in the lesions, or to transmit the condition experimentally by inoculation or contact were unsuccessful. He reported that a familial occurrence of the lesion had been observed repeatedly, suggesting the possibility of congenital or hereditary predisposition.

The name of Crohn's disease was given to terminal ileitis in man following the work of Crohn, Ginzburg and Oppenheimer (1932). Two forms are recognised. An acute form is seen most often in young persons, in whom it may be mistaken for acute appendicitis. In these cases the terminal part of the ileum is found to be swollen and hyperaemic, with patches of fibrinous exudate on the surface of the gut and oedema of the mesentery. The maximum frequency of this form occurs in children under ten years of age (Strömbeck, 1937). The chronic form is encountered at any age, with a maximum frequency in the age group 20 to 30 years. It is characterised by a thickening of the terminal section of the ileum, which becomes thick-

*Veterinary Investigation Centre.

walled and stiff as a garden hose. Acute peritonitis resulting from perforation is a rare complication. The etiology of the disease in man is still obscure. A familial occurrence of the lesion has been mentioned in some cases.

Crohn's disease is a well-recognised clinical entity in Great Britain but terminal ileitis has not been previously reported in swine in this country. The purpose of the present communication is to record the recognition in East Anglia of a condition in young pigs similar to that described by Emsbo.

CLINICAL HISTORY OF HERD

The total breeding stock consisted of 16 sows and one boar, but so far as is known, losses were confined to the progeny of one sow and two gilts. The gilts were litter sisters but were unrelated to the sow. The herd was established in 1947 and the breeding and rearing of pigs had been carried on without untoward losses until March 1951, when four members of the litter of a sow died when 2 to 3 months of age. All four pigs showed peritonitis at post-mortem examination. A fifth member of this litter died about four weeks later. This sow has since had two further litters by the same boar and the pigs were reared without loss.

Sporadic losses occurred between March 1951 and February 1952, four young pigs dying from ileitis during this period. As a detailed record of the losses was not kept it is not known whether these pigs came from the same or different litters.

In February 1952, losses occurred in the progeny of the two litter sisters. Both gilts had been mated to the same boar and farrowed on the same day. Losses commenced after weaning when the pigs were 12 to 13 weeks of age. Three died in one litter and two in the other; of these, three were examined in the laboratory and two by the owner's veterinary surgeon. All showed ileitis and peritonitis at post-mortem examination. The 14 survivors from the two litters were sold to another farm for fattening. Within a few days of purchase two of the pigs died from ileitis after a short illness. There is evidence, therefore, that 12 pigs in the three litters died from this disease at 3 to 4 months of age. The remaining pigs were fattened to bacon weight and 5 of these were examined at slaughter when 7 to 8 months of age. Three showed typical gross lesions of ileitis and the remaining two showed histo-pathological lesions resembling those seen in this disease (Pigs 72 to 76 inclusive).

FEEDING AND MANAGEMENT

Pregnant females were allowed to run at pasture to within a fortnight of farrowing, when they were brought into farrowing pens. Provided the weather was suitable, litters were given a daily run at pasture from about 1 to 3 weeks of age. Piglets reared indoors were creep fed from the time they were 3 weeks of age, but this practice was discontinued in the winter of 1951-52. Wet feeding was prac-

tised and one brand of proprietary meal was fed to the breeding stock and the younger weaned pigs. Of the three affected litters, two were reared intensively, and the other was turned out to pasture when 3 weeks old.

SYMPTOMS

The symptoms in each fatal case were those of acute peritonitis. The illness was sudden in onset and of short duration. Affected pigs showed loss of appetite, dullness and a disinclination to get up when disturbed. Although the pigs appeared anxious to feed and ran to the trough, they ate very little food. Excessive thirst was a prominent symptom. Breathing was distressed and all the pigs examined showed a sub-normal temperature. A bluish discoloration of the skin was present in most cases. The appearance of symptoms was followed by a progressive depression terminating in death in from 24 to 48 hours.

LABORATORY EXAMINATIONS

Macroscopic changes

Case No. 1. Reflection of the abdominal wall showed a diffuse, foetid peritonitis. The intestines were extremely friable and were separated with difficulty. The extreme thickness of the terminal portion of the ileum immediately caught the eye, the thickening extending for some 70 cm. and gradually diminishing towards the jejunum. Closer examination showed a perforation of the thickened ileum towards its anterior end, from which intestinal contents had escaped into the peritoneal cavity. An incision into the thickened portion of the ileum showed the mucous membrane to be raised into irregular folds, the lumen being almost occluded (Fig 1). Cultural examination of the viscera was negative for any bacterial infection.

Case No. 2. The pig showed some diffuse peritonitis as in the previous case. The terminal part of the ileum did not show the same degree of "garden hose" thickening but was, nevertheless, larger than normal; the mesenteric lymphatic glands were also enlarged and oedematous. *C. pyogenes* was isolated in culture from the mesenteric glands, the spleen and liver.

Case No. 3. This pig more nearly resembled Case No. 1. Reflection of the abdominal wall again showed a diffuse, foetid peritonitis. There was gross enlargement of the terminal part of the ileum with perforation, and enlargement of the mesenteric lymph glands. *C. pyogenes* was isolated in culture from the peritoneal contents, the mesenteric, gastric and hepatic lymph glands, and from the liver and spleen.

Examination of Abattoir carcasses

Of the five pigs which were examined at the bacon factory, three showed enlargement of the last part of the ileum. The pigs were in good condition and showed no macroscopic lesions elsewhere. The thickening of the ileum, which was most marked at the ileo-caecal junction and diminished towards the jejunum, could be detected

easily by running the intestines through the hands. The degree of thickening was in no case as marked as in the clinical cases. Figure II shows the degree of thickening of an affected portion of intestine; for comparison a piece of normal ileum from a pig of the same age has been included.

Histopathology

Examination of representative pieces of tissue from eight cases of the disease showed a similarity in the type of reaction but variation in its degree. The inflammatory reaction was essentially sub-acute or chronic, usually with superimposed acute inflammatory changes due to the terminal peritonitis.

In six of the pigs the mucous surface of the intestine was intact despite a catarrhal inflammation and the presence of an excess number of goblet cells. Pig No. 72 and Case No. 2, however, showed ulceration of the mucosa due to what appeared to be fairly extensive infarction. The infarcts were necrosed and there was surprisingly little reaction at the periphery of the dead tissue. The ulceration and necrosis did not extend beyond the muscularis mucosae.

The most striking feature of all the cases was the extreme degree of cellularity of the mucous membrane and the sub-mucosa, which is well shown in the photomicrographs (Figs. III and IV). The infiltrating cells were mainly of the mononuclear series and comprised plasma cells, lymphocytes and epithelioid cells in that order of frequency. Eosinophils were also present and indeed in pigs Nos. 75 and 76 they were the predominant cell. The villi of the mucous membrane had been distorted by the massive cellular infiltration. In some areas scarcely any glandular tissue was discernible due to its replacement by masses of cells (Fig. IV) In other areas the accumulations of cells had produced finger-like projections of the mucosa which protruded into the lumen of the gut. The cellular infiltration was quite diffuse and the invading cells were not arranged in any orderly fashion. No giant cells were seen and nothing in the nature of a "tuberculous follicle" was detected.

The muscularis mucosae was much thickened in Case No. 2. Here the increase in thickness was due to deposition of collagen and not to muscular hypertrophy or hyperplasia. In other cases, the muscularis mucosae was not easily recognised and the mucous membrane and the submucosa appeared to be continuous. The sub-mucosa was infiltrated with the same types of cell as were present in the mucous layers except that fibroblasts were rather more frequent. In case No. 2, there were areas of early granulation tissue. The lymph follicles were very prominent and showed hyperplastic changes with hyperchromatic cells. Many of the follicles contained plasma cells and in some of the very reactive follicles Russell bodies were conspicuous. Around the follicles were varying amounts of collagen.

Changes were noted in the muscular layers of the gut. These changes were all degenerative and were related to the peritonitis. This was of the usual acute fibrinous variety and does not require any detailed description.

The lymph nodes were examined in some of the eight cases of regional ileitis. The tissue reaction was not considered specific but all the nodes showed hyperplastic changes and two of them exhibited an acute lymphadenitis. The congestion, haemorrhage, exudation and sinus catarrh were directly referable to the acute peritonitis.

DISCUSSION

In the human subject the earliest histological change appears to be a hyperplasia of the lymph follicles with marked oedema of the submucosal tissue and infiltration with round cells. A more specific change is then seen in the development of giant cell systems. These are formed by the accumulation of proliferating epithelioid cells together with giant cells of the Langhans type. Such lesions have a marked similarity with tubercles but no caseation occurs. In the later stages of the human disease secondary infection occurs which may lead on to ulceration, fissures, cracks, abscesses and fibrosis.

Emsbo's cases differed in several respects from those described in man. He noted diffuse anaplastic changes in the superficial and glandular epithelium and also a reduction or cessation of mucus production. A considerable degree of downgrowth of epithelium giving the impression of a "malignant adenoma or adenocarcinoma" together with "epithelial metastases to the ileo-caecal lymph node" and the formation of "typical Leiberkuhn's glandular tubes with well developed mucus-secreting goblet cells" were changes described by Emsbo which differed from those seen in human cases.

In the present investigation, although the gross pathology appeared identical with human ileitis the close similarity was not maintained in the histopathological changes. Unlike Emsbo we were unable to find the giant cell systems which form such a feature of the human condition. The diffuse cellular infiltration, the lymphoid hyperplasia and the changes in the muscular coats were as seen in human and Emsbo's porcine cases. The epithelial anaplastic changes and the metastases to the regional lymph node described by Emsbo were absent from our cases.

Despite the differences in the histopathological changes between human and porcine ileitis, there is an interesting relationship in the age incidence at which the disease appears. In man the maximum frequency of the acute form lies in the group under 10 years of age, while in pigs acute ileitis with peritonitis is seen most commonly in pigs under 3 months of age. The chronic form in man is encountered at any age with a maximum frequency in the age group 20 to 30 years. In swine this type is frequently seen when pigs are slaughtered for bacon at 7 to 8 months of age. It seems probable

therefore, that both in man and in swine, ileitis develops early in life. The early, acute, clinical cases may be a reflection of the severity of the condition in the individual, complicated, in the case of young pigs, by perforation and peritonitis. If complications do not occur, the lesion remains chronic, possibly throughout life. This similarity in the age incidence in the human and pig respectively suggests a common etiological factor. The isolation of *C. pyogenes* from two of our three clinical cases is probably of no etiological significance, as this organism is a common secondary invader in swine.

In the present observations no attempt was made to transmit the condition as suitable animals were not available for experimental purposes. No cell inclusions were detected in the lesions to suggest a virus infection.

The disease in pigs appears to be familial only in the sense that practically all members of a litter may be affected. In our cases, 12 of the 27 pigs in the three litters died from ileitis with peritonitis and a further 5 showed ileitis when slaughtered at bacon weight. The fact that one sow has subsequently reared two normal litters sired by the same boar tends to exclude the possibility of congenital or hereditary predisposition.

SUMMARY

An account is given of an outbreak of regional ileitis in pigs, in which the clinical symptoms, post-mortem and histo-pathological findings were in many respects similar to those described by Emsbo (1951). The histopathology is described and compared with that found in human ileitis (Crohn's disease). Attention is drawn to the close relationship in the age incidence of acute and chronic ileitis in humans and pigs respectively. The suggestion is made that the etiology of these conditions may be similar.

REFERENCES

- Crohn, B. B., Ginzburg, L., and Oppenheimer, G. D. (1932). *J. Amer med. Ass.*, **99**, 1923.
Emsbo, P. (1951). *Saertryk. af. Nord. Vet. Med.*, **3**, 1.
Strombeck, J. P. (1937). *Acta Chir. Scandinav.*, *Suppl.* 50, 1.

LEGENDS TO PLATES

- FIG. I. Shows the typical thickening of the ileum.
Note the folds and corrugations of the mucosa.
- FIG. II. Ileum from normal pig and a thickened gut from a case of regional ileitis.
- FIG. III. Massive cellular infiltration of mucosa and submucosa. Catarrh of the mucous lining. H/E. x 80..
- FIG. IV. Replacement of glandular tissue by cellular masses. This is a higher magnification of Fig. III x 200.

FIGURE I.

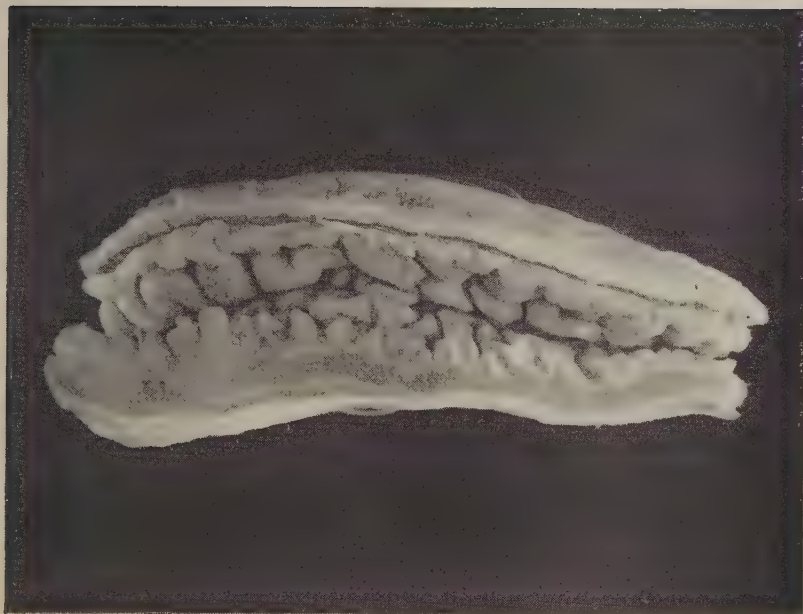


FIGURE II.

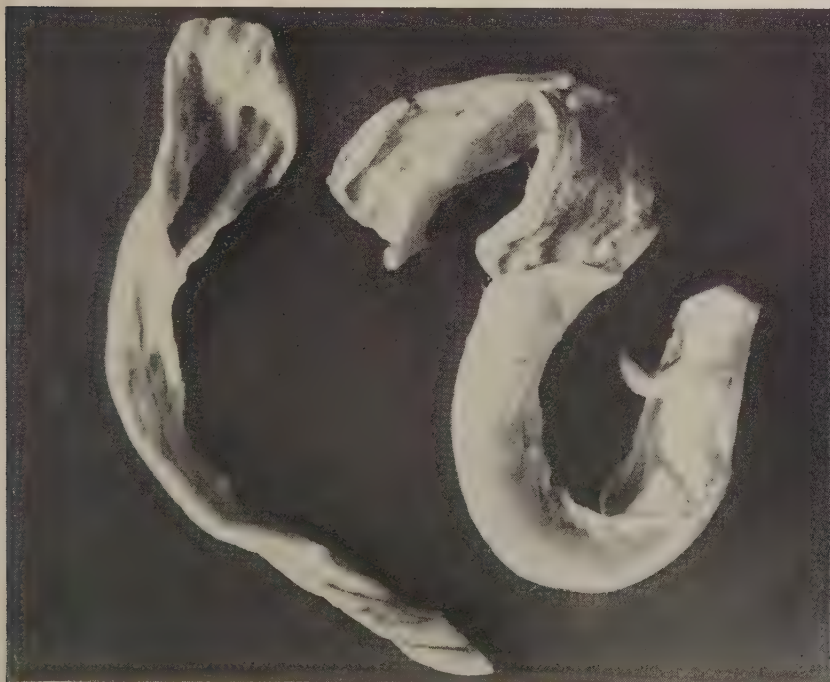


FIGURE III.

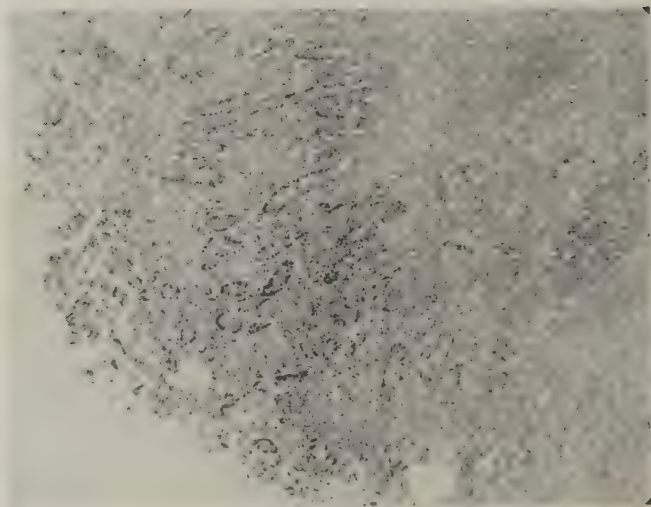
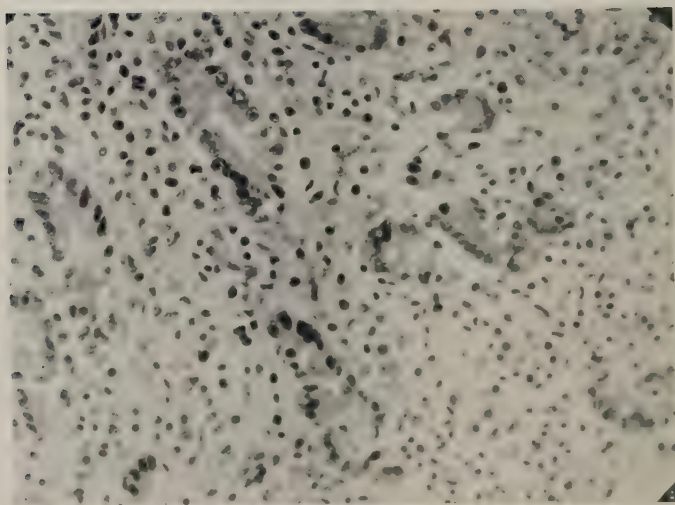
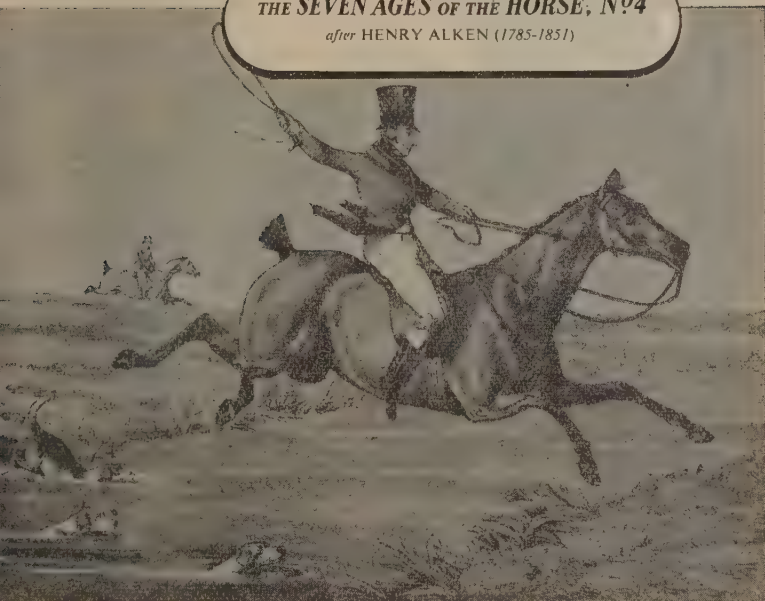


FIGURE IV.



THE SEVEN AGES OF THE HORSE, N^o4

after HENRY ALKEN (1785-1851)



*Then the Hunter, his sinews stronger grown,
and dock of Melton cut, jealous of being
first sudden and quick in action, seeking
the bubble reputation even at a seven yards break.*

Reproduced by courtesy of Fores Ltd., London, W.1.

In penicillin therapy **'DISTAQUAINE'**
brand
preparations of procaine penicillin offer the
following advantages:

- ★ Aqueous, containing neither oil nor wax
- ★ Easy to prepare and administer
- ★ Least possible pain on injection
- ★ Effective blood levels up to 24 hours
following administration
- ★ Dry syringe unnecessary
- ★ Equipment easily cleaned after use

'DISTAQUAINE' SUSPENSION
vials of 10 ml. (300,000 units per ml.).

'DISTAQUAINE' G
vials of 300,000, 900,000 and 3,000,000 units.

'DISTAQUAINE' FORTIFIED
vials of 400,000 and 1,200,000 units.

available from:

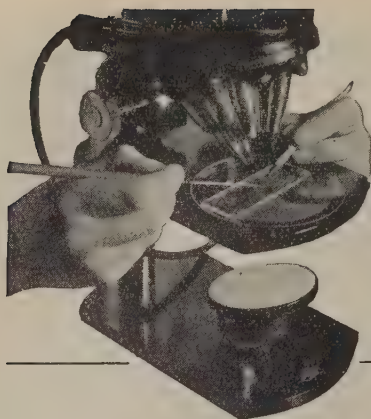
Allen & Hanburys Ltd.,
British Drug Houses Ltd.,
Burroughs Wellcome & Co.,
Evans Medical Supplies Ltd.,
Imperial Chemical (Pharmaceuticals) Ltd.,
Pharmaceutical Specialities
(May & Baker) Ltd.

'Distaquaine', a trade mark, is the property of the manufacturers

THE DISTILLERS COMPANY
(BIOCHEMICALS) LIMITED

SPEKE

LIVERPOOL



in the war against **BACTERIA**

'CRYSTALLINE' PENICILLIN G

'Avlon' brand. Available as the sodium salt, for use wherever penicillin of the highest purity and potency is required.

Penicillin 'AVDET' Teat Bougies

Specially designed for the treatment of mastitis, each 'Avdet' contains 25,000 units of potassium penicillin G in a non-irritant, milk-soluble base.

'UDOLAC'

A potent yet safe antibacterial drug of exceptional value in the treatment of streptococcal mastitis.

'CETAVLON'

A powerful bactericide and detergent, 'Cetavlon' has a wide range of uses in veterinary practice. Two special formulations have been designed particularly for use in preventing the spread of mastitis.

'SULPHAMEZATHINE'

Highly effective against a wide range of Gram-negative and Gram-positive organisms. A single dose daily is adequate in the treatment of all species but the dog.

also 'Avlon' brand

**SULPHANILAMIDE
SULPHAGUANIDINE
SULPHANILAMIDE
with Proflavine Hemisulphate**

**ACRIFLAVINE
EUFLAVINE
PROFLAVINE HEMISULPHATE**

IMPERIAL CHEMICAL (PHARMACEUTICALS) LTD.

A subsidiary company of Imperial Chemical Industries Ltd.

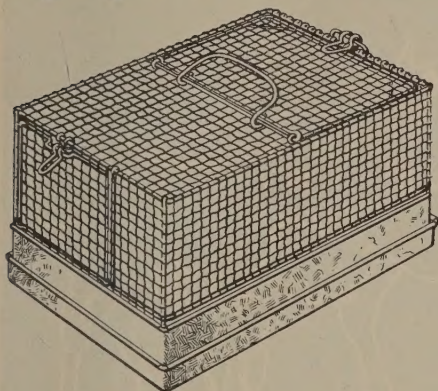
Wilmslow, Manchester



Ph. 184

**Complete
Equipment for**

ANIMAL HOUSES



Specialists in
**CAGES and RACKS
for Rats, Rabbits,
Guinea Pigs, etc.**

On the Lists of M.R.C., A.R.C.
and the Leading Hospitals and
Universities:

*Send us particulars of your
requirements.*

Deal with the actual Manufacturer.

S. RAMSEY & COMPANY LIMITED

Sebastian House - Sebastian Street - London, E.C.1

H. K. LEWIS & Co. Ltd.

MEDICAL BOOKSELLERS and PUBLISHERS
BOOKSELLING DEPARTMENT. Very large Stock of Textbooks
and Recent Literature in all branches of Medicine and Surgery of all
Publishers. Catalogues on request. Please state interests.

FOREIGN DEPARTMENT. Select stock of Medical and Scientific
Works. Books not in stock obtained from U.S.A. and the Continent
under Board of Trade Licence at the most favourable rates.

MEDICAL STATIONERY DEPARTMENT. Case-Taking Sys-
tems (Cards or Sheets), Temperature and other Charts, etc.

SECOND-HAND DEPARTMENT. 140 GOWER STREET.
Large Stock of **Second-Hand** recent editions always on view.
Old and Rare Books sought for and reported when found.

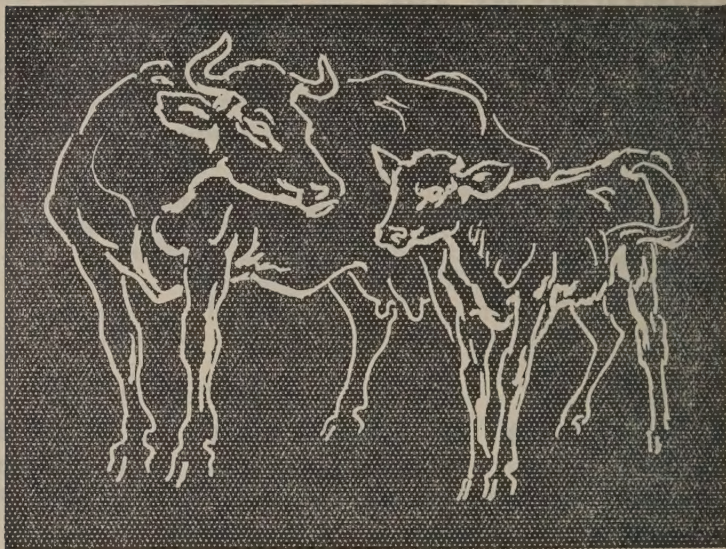
LENDING LIBRARY—Medical and Scientific
ANNUAL SUBSCRIPTION from TWENTY-FIVE SHILLINGS

PROSPECTUS POST FREE ON REQUEST

THE LIBRARY CATALOGUE revised to December, 1949, containing
classified Index of Authors and subjects. To Subscribers 17s. 6d. net; to
Non-Subscribers 35s. net, postage 1s. 3d.

LONDON : H. K. LEWIS & Co., Ltd.,
136, GOWER STREET, W.C.1.
Telephone : EUSTON 4282 (7 lines)

FOR UTERINE ASEPSIS



Field reports indicate that 'Compron' medication is superior to that offered by older types of pessary. These compressed products are designed to enable the relatively rapid solubility and absorption of sulphanilamide to be supported 'in utero' by the prolonged action of the more slowly absorbed sulphapyridine. The reliable and rapid disintegration of 'Compron' ensures the ready availability of these sulphonamides and their dispersal over the surface of the uterine mucosa. 'Compron' is indicated in both cows and mares in cases of dystokia, retained placenta, etc.

Supplies: Boxes of 10. Further information will be supplied by our Veterinary Division on request.

'COMPRON'

trade mark

brand

COMPRESSED PRODUCTS OF
SULPHANILAMIDE AND SULPHAPYRIDINE

TO THE PROFESSION ONLY



manufactured by

MAY & BAKER LTD

'phone: ILFord 3060 Exts. 24 & 236

VA161

DISTRIBUTORS: PHARMACEUTICAL SPECIALITIES (MAY & BAKER) LTD · DAGENHAM

THE BRITISH VETERINARY JOURNAL

(Established 1875)

A MONTHLY REVIEW OF VETERINARY SCIENCE

RECENT CONTRIBUTIONS *include the following papers*

- T. GRAHAME—The Pelvis and Calyces of the Kidneys of some Mammals.
J. RUSSELL GREIG—The Sheep Dog, Its Management and Feeding.
A. M. HARTHOORN—The Case for a practical approach to the Problem of Shock.
S. ISHII, A. YAJIMA, Y. SUGAWA and others—The Experimental Reproduction of so-called Lumbar Paralysis—Epizootic Cerebrospinal Nematodiasis—in Goats in Japan.
S. E. PIERCY—Studies in Bovine Malignant Catarrh.
W. PLOWRIGHT—Observations on the Behaviour of Rinderpest Virus in Indigenous African Sheep.
G. T. WILKINSON—The Pathology of Navicular Disease.
F. W. WITHERS—Mortality Rates and Disease Incidence in Calves in relation to Feeding Management and other Environmental Factors.

Annual Subscription 40s

Published by:

BAILLIÈRE, TINDALL & COX LTD.

8 Henrietta Street, Covent Garden, London, W.C.2

GURR'S



Established 1915

**STANDARD STAINS AND
REAGENTS FOR MICROSCOPY
KNOWN AND USED**

**THROUGHOUT
THE WORLD**

Write for Catalogue A15

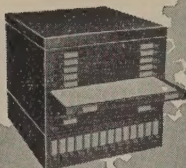
NOW ready

**'BIOLOGICAL
STAINING METHODS'**

5th Edition, considerably enlarged;
'the most complete manual of its kind'
Illustrated in colour. 5/6 post free.

**GEORGE T. GURR LTD.
136 NEW KINGS ROAD
LONDON, S.W.6**

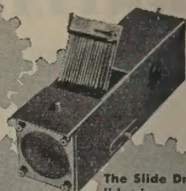
more than a mere laboratory instrument



The Block File stores paraffin blocks with utmost economy of space



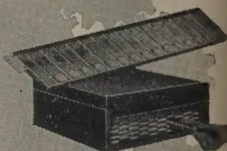
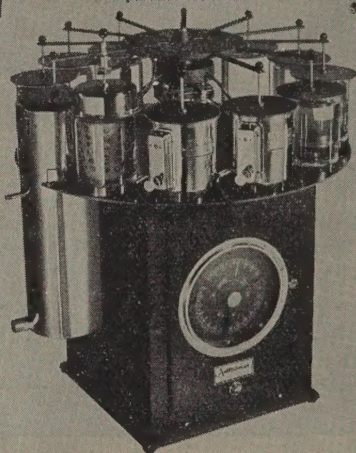
The constant-temperature Water Bath for floating paraffin sections



The Slide Dryer dries slides in seven minutes



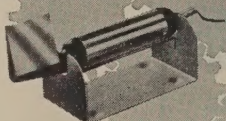
The Slide-files for all types of slides and transparencies



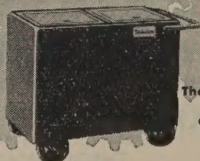
The Laboratory Slide Trays and Cabinet



The standardized reagents for tissue processing and staining



The electric Paraffin Knife for cutting, blocking and mounting tissues



The Mobile Refrigerator for gathering and storing tissues

**an integrated system for every phase
of histologic tissue preparation and reference**

Autotechnicon

Trade Mark Registered U. S. Patent Off.

**automatic tissue processing
by any histologic technique**



The Autotechnicon System, as employed in a typical hospital working day, is interestingly discussed in an informative brochure. A copy will be sent on request to . . .

In thousands of hospitals, the world over, the benefits of the Autotechnicon System of mechanized tissue-processing procedures are being demonstrated daily. Production is improved, time saved, efficiency heightened, labor conserved. The Autotechnicon System is unique . . . the outgrowth of decades of intimate study of, and experience with, the technical problems of the pathologic laboratory.

Address in U.K.: 7 LYTTON GARDENS, WALLINGTON, SURREY